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INFLUENCE OF HUMIDITY UPON THE STRENGTH AND *THE ELASTICITY OF WOOL FIBER*[1]

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INTRODUCTION

Much work has been done on the study of the fiber, the yarn, and the finished cloth of wool. It has long been known that wool absorbs moisture from the air, but the first real research along this line appeared by Schloesing (*7*)[2] in 1893. This work was upon the relation of the moisture content of clean wool to the humidity of the air. In 1905 Hartshorne (*2*) published work along this line, the results of which were in substantial agreement with the work of Schloesing. Hartshorne (*3*) formulated his results into the "The laws of regain in cotton and worsted," using these laws in the construction of tables showing the moisture content of wool for a wide range of moisture and temperature conditions of the atmosphere. These tables show the great delicacy with which wool responds to the changes in the relative humidity of the air, and also makes it easy to find the moisture content of wool where the relative humidity of the air is known. He has continued his work upon the regain of worsted and of cotton and is to-day one of our greatest authorities along this line.

The effect of moisture on the strength and elongation of yarns and fabrics was reported by Barker, Barbrick, and Pickles (*1*). In their tests on worsted yarn they found that on increasing the moisture content from "absolute dryness" to saturation there was a decrease in strength but an increase percentage of elongation. They also found that when like patterns of worsted were tested in a room of 92 per cent humidity and then in a room with a humidity of 76 per cent there was an increase in strength and a decrease in elongation. They further found that yarns or fabrics made of cotton increased in both strength and elongation on the increase of the humidity of the surrounding atmosphere.

[1] Approved for publication in the Journal of Agricultural Research by the Director of the Agricultural Experiment Station of the University of Wyoming.

[2] Reference is made by number (italic) to "Literature cited," p. 294-295.

Lewis (5) made tests on woolen and worsted yarns, similar to those of Barker and his coworkers, under controlled conditions of temperature and humidity, at five different humidities ranging from 45 to 85 per cent. He found an increase of 16 per cent in the tensile strength of cotton and a decrease of 18 per cent in the tensile strength of worsted for a rise of 40 per cent in the relative humidity.

The work carried on at the Wyoming Experiment Station in 1911 under the direction of Hill (4) showed that the dry wool fiber was stronger than the wet fiber, and that at a humidity of approximately 15 per cent the wool fiber was stronger than at 35 per cent. Because of the lack of the means of temperature and humidity control, this work was temporarily suspended until such control conditions might be established.

EXPERIMENTAL WORK

On undertaking research studies upon wool the writer found that it was first necessary to improve further the means of measuring the strength of the wool fiber before a continuation of studies in the effects of chemical reagents and of alkali and weathering could be made with satisfactory results. In September, 1917, the writer succeeded in bringing a small inside room under automatically controlled conditions of temperature and humidity. A description of this room will be found elsewhere in this article. The work of Hill (4) who tested over 59,000 fibers, clearly showed that it was quite impossible to get satisfactory results by testing the single wool fibers under ordinary room conditions. He states (p. 123):

> The variation of the means of hundreds is so great that the mean of this or a smaller number of tests is a very inaccurate measure of the mean of a sample of wool containing only a few thousand fibers, and that the means of thousands can scarcely be used for anything more than the most general work.

Anyone who has tested textile fibers knows that to test only 500 wool fibers is not only a long but a tedious operation, and it would be impracticable to test many samples, were so many tests required for each sample. It was thought, however, that possibly under controlled conditions of temperature and humidity the number of fibers necessary to be tested on each sample, with satisfactory results, might be greatly reduced. With this thought in mind the writer began the work covered in this paper, with a plan outlined to test samples of wool fibers at five humidities ranging from 40 to 80 per cent. Samples of wool from the shoulders of four sheep, a Rambouillet, an Oxford, a Cotswold, and a Dorset were selected. All tests were made upon single fibers from locks of wool which had not been cleaned or scoured. The tests were all made on a Reeser and Mackenzie fiber-testing machine, a machine devised by Matthews, of the Philadelphia Textile School, and fully described in Matthews's Textile Fibers (6, *p.* 254).

In these experiments and all subsequent work the temperature was kept at 70° F., the humidity only being changed. The breaking strengths of the 200 fibers were determined on each sample, first at a humidity of 40, and later at a humidity of 70 per cent. The results of this work are shown in Table I.

TABLE I.—*Breaking strength of wool fibers at two humidities*

Sample No.	Breed.	Relative humidity, 40 per cent.			Relative humidity, 70 per cent.		
		Average of—		Variation between 100 and 200.	Average of—		Variation between 100 and 200.
		100.	200.		100.	200.	
		Dgm.	*Dgm.*	*Per cent.*	*Dgm.*	*Dgm.*	*Per cent.*
991	Rambouillet	70.82	71.04	0.62	63.14	62.68	1.50
991	do	71.26	.....	.....	62.22	.....	.....
994	Oxford	149.49	163.30	15.59	150.74	152.80	2.65
994	do	177.11	.....	.....	154.86	.....	.....
996	Cotswold	169.86	178.80	9.56	182.00	178.70	3.63
996	do	187.81	.....	.....	175.40	.....	.....
997	Dorset	148.14	140.18	10.74	130.44	130.73	0.44
997	do	132.22	.....	.....	131.01	.....	.....

An examination of Table I, shows that with the increasing of the humidity the breaking strength of the fibers decreases. It will also be noted that the percentage variation between the average breaking strengths of each hundred fibers reaches in one case practically 16 per cent. Had a larger number of fibers been broken, it is probable that the extreme variations between hundreds would have been even greater.

Determining the breaking strength of the fibers under controlled conditions of temperature and humidity is more accurate than under ordinary room conditions; yet the wide variations among the sizes of the individual fibers makes it quite impossible to obtain a small percentage variation between the means of each hundred fibers tested without taking into consideration the diameter of the fibers. An attempt was made to measure the diameter of the fibers in the testing machine by means of a microscope which could be moved horizontally or vertically by means of a screw adjustment. The work was found very slow and tedious, and it appeared that the fibers did not break at the smallest diameter. The fact that wool fibers are very irregular in shape renders the measurements taken from one side of the fiber very inaccurate. If a microscope can be constructed to view the wool fiber from two different angles at the same cross section, there may be obtained much more accurate results by use of this instrument. It seems that this condition may be obtained by a proper adjustment of mirrors, but to the writer's knowledge no such adjustment has ever been tried.

The next arrangement which suggested itself as a means of measuring the fibers was the use of a micrometer caliper. A micrometer caliper graduated to read in hundredths of a millimeter and having a ratchet stop adjustment can readily be set so that contact upon the fibers is uniform and the fiber is not distorted when the contact is made. This micrometer is substituted in place of the lower jaw of the testing machine (Pl. 48, A) so that the diameters may be measured with the greatest speed and accuracy possible with a micrometer. A small hand lens (not shown in the illustration) was supported in front of the micrometer in order to make it possible to read the diameters of the wool fibers to a thousandth of a millimeter.

The diameters of a number of fibers were measured at as many intervals as possible between the two jaws of the testing machine, after which the fibers were tested. The fibers broke in practically every instance at the place where the micrometer indicated the smallest diameter. A number of fibers were very carefully watched under a hand lens as they were being measured with the micrometer. It was observed that as the contact is being made the oval fibers twist so the measurement is made at the smallest diameter. Human hair and the hair from animals were tested with the same result. This led the writer to believe that he was justified in using the smallest diameters obtained by the use of a micrometer in computing the tensile strength (ratio of breaking strength to area of cross section) of the wool fibers.

Another series of tests was made on the same samples as reported in Table I, but for five relative humidities, 40, 50, 60, 70, and 80 per cent, temperature 70° F. Every wool fiber tested was measured at three places between the jaws of the testing machine. The stretch of each fiber was recorded, together with its breaking strength, and the tensile strength calculated from the diameter of the fiber as found at the smallest point. The results of the measurement of the breaking strength are shown in Table II.

TABLE II.—*Breaking strengths of fibers at five humidities*

Sample No.	Breaking strength at a relative humidity of—									
	40 per cent.		50 per cent.		60 per cent.		70 per cent.		80 per cent.	
	Average of 100.	Average of 200.	Average of 100.	Average of 200.	Average of 100.	Average of 200.	Average of 100.	Average of 200.	Average of 100.	Average of 200.
	Dgm.	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*
991	66.82	67.17	65.39	69.92	67.50	69.45	54.67	55.85	55.36	53.85
992	67.52	.....	66.44	.....	71.40	.....	57.02	.....	52.34	.....
994	182.07	187.49	140.19	146.26	145.12	135.77	159.43	162.12	120.89	129.93
994	194.70	.....	152.33	.....	126.42	.....	164.80	.....	138.96	.....
996	172.59	195.18	196.67	206.21	248.88	229.90	210.79	200.72	192.72	175.75
996	217.77	.....	215.74	.....	209.12	.....	190.64	.....	158.79	.....
997	124.53	125.18	102.45	105.44	102.06	105.29	104.10	106.85	103.30	100.20
997	125.70	.....	108.42	.....	108.53	.....	108.35	.....	97.10	.....

TABLE III.—*Diameter, breaking strength, and tensile strength of wool fibers at five different humidities*

Sample No.	Relative humidity of—																			
	40 per cent.				50 per cent.				60 per cent.				70 per cent.				80 per cent.			
	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100.)	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.
		Mgm.	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*
991	15.5	6,751	357.7	363.1	16.1	6,539	325.2	323.8	16.9	6,750	280.6	290.2	16.0	5,702	283.6	276.1	15.7	5,234	270.4	278.2
991	15.2	6,682	368.5		16.2	6,644	322.4		18.0	7,140	299.7		16.1	5,467	268.6		15.7	5,536	286.0	
994	27.7	19,470	323.1	323.0	23.7	14,019	317.7	315.3	24.1	12,642	277.2	275.3	28.6	16,480	256.6	264.5	25.8	13,896	265.8	255.1
994	26.8	18,208	322.8		24.9	15,233	312.8		26.1	14,512	273.4		27.3	15,943	272.4		25.1	12,089	244.3	
996	29.1	21,777	327.4	332.7	29.2	19,667	293.7	300.5	30.3	20,912	290.1	278.1	29.3	19,064	282.8	283.8	29.8	19,271	276.3	269.0
996	25.5	17,259	337.9		29.9	21,574	307.3		34.5	24,888	266.2		30.8	21,079	282.9		27.8	15,879	261.6	
997	25.2	12,453	249.7	242.3	23.0	10,246	246.6	243.1	25.6	10,853	212.5	219.1	25.5	10,835	212.1	213.0	26.6	10,330	185.9	189.5
997	26.1	12,570	235.0		24.0	10,842	239.7		24.0	10,206	225.6		24.9	10,410	213.9		25.3	9,710	193.2	
Average	......	......	......	315.3	......	......	......	295.7	......	......	......	265.7	......	......	......	259.3	......	......	......	242.9

Table II shows what a large variation may occur in the averages of the breaking strengths of 100 fibers. In the case of No. 991, the fibers are fairly uniform, and there is less variation. The variations are so great in most cases that one would not be justified in making any final deductions from the results. The differences which occur in the breaking strengths of different fibers in the same sample may be more clearly seen by comparing the results of humidities 40 and 70 in Table II with similar

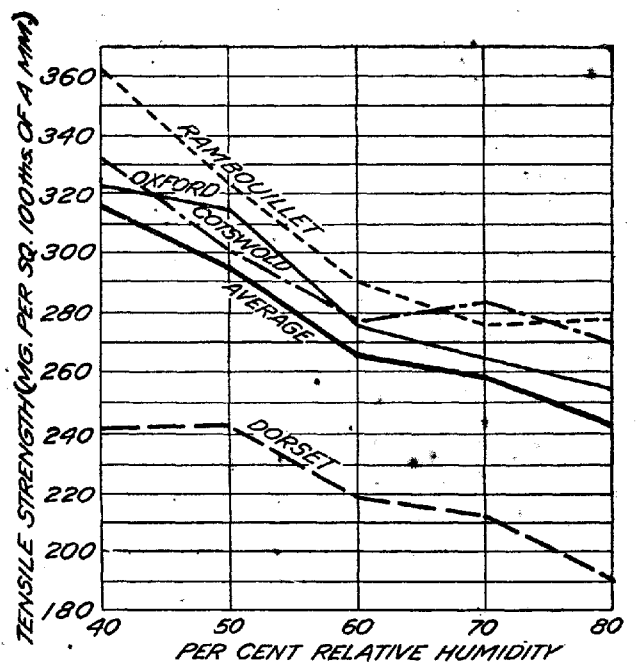

FIG. 1.—Graphs showing the effect of humidity upon the tensile strength of the wool fiber.

results upon the same sample as shown in Table I. Such large variations will occur when the size of each fiber is left out of consideration.

Table III shows the average diameter, breaking strength, and tensile strength for 100 fibers and the average tensile strength for 200 fibers. It can readily be seen that when the diameters of the individual fibers are taken into consideration there is much more uniformity in the results obtained. Had 500 fibers been tested, it is no doubt true that the average tensile strength would have been somewhat more accurate than when only 200 fibers were tested. In the case of sample 991 (humidity 70) 600 fibers were broken, and the average tensile strengths for each

hundred tested were, respectively, 269, 273, 275, 280, 283, and 288. The smallest and largest averages obtained from any two of these figures are 271 and 286, respectively. If each sample is taken into consideration, it will be observed that the average tensile strength is greater in every case at a humidity of 40 than at 60, 70, or 80 per cent. It will also be noted that the average tensile strength of every sample is greater at a humidity of 50 than at 70 or 80, and at 60 it is greater than at 80 per cent. The tensile strength decreases with the increase in the humidity, although in some cases there may be a slight variation up or down when the sample tested is compared with the one tested at the next higher or lower humidity. The average tensile strength of four samples at the different humidities gives figures which show a direct ascent as the percentage of relative humidity is reduced. It would seem that if an average of the four samples was taken, the effects of humidity upon the tensile strength of the wool fiber could be more clearly seen. Graphs of these averages are given in figure 1.

It is again clearly noted that there is a direct increase in the tensile strength of the wool fiber as the relative humidity is reduced, and vice versa. The presence of more yolk on one fiber than on another would make an added variation, as would also the percentage error in the measurement of the fibers.

The percentage elasticity of these four samples was determined at the same time as their breaking strengths, the results being given in Table IV. These tests show that the wool fiber increases in elasticity as the humidity increases. Figure 2 shows curves plotted from the average elasticity of each of the four samples for each humidity, together with the average of all.

It seems probable that each sample would show a curve in closer agreement with that of the final average of figure 2 had 500 or 1,000 fibers been broken upon each sample at the different humidities.

TABLE IV.—*Percentage elasticity of wool fibers at five humidities*

Sample No.	Number of fibers tested.	Elasticity at a relative humidity of—				
		40 per cent.	50 per cent.	60 per cent.	70 per cent.	80 per cent.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
991	200	28.02	31.74	33.67	34.88	35.52
994	200	30.78	33.32	36.92	39.60	42.41
996	200	32.76	38.38	40.24	41.26	47.02
997	200	19.68	26.30	26.44	28.64	31.38
Average		27.81	32.44	34.32	36.10	39.08

The present paper is a progress report, and further humidity studies are being made, both with raw and clean wool.

TEMPERATURE AND HUMIDITY CONTROL

The question of automatically controlling the temperature and humidity is perplexing to the Experiment-Station worker whose funds

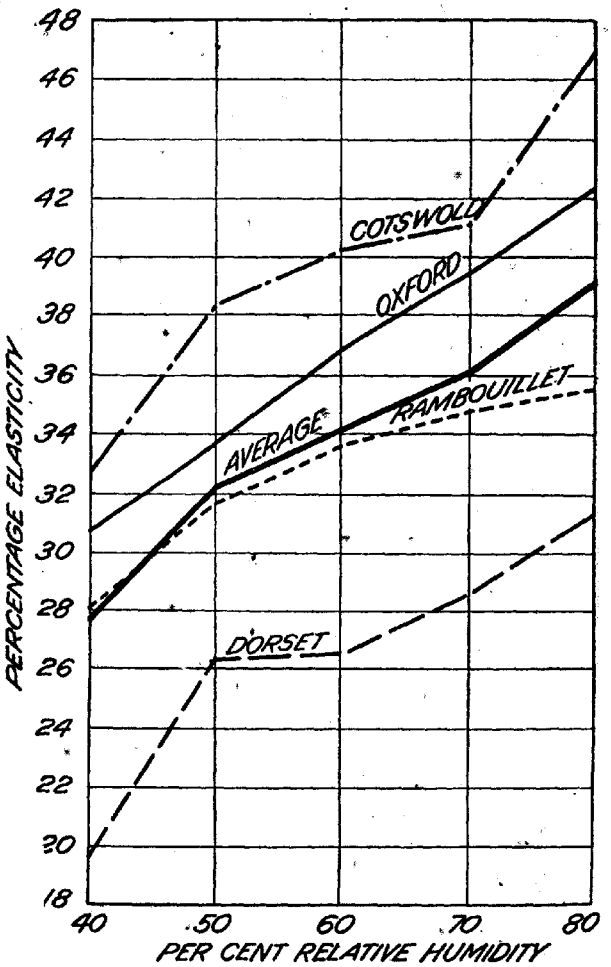

FIG. 2.—Graphs showing the effect of humidity upon the elasticity of the wool fiber.

are limited. The humidity room operated by the writer is simple in its method of control and can be readily installed at a minimum cost.

The room used is an inside one, 6 feet wide, 6 feet long, and 12 feet high. The walls are made of hollow 4-inch gypsum blocks plastered

on both sides, and the ceiling and floor of reinforced concrete. In one side there is a large, well weather-stripped window, the upper part of which has an opening 7 inches wide and extending the entire width of the window. This opening was designed for ventilation purposes, but was found inefficient and was displaced by artificial ventilation. The entrance to the room is provided with double doors separated from each other by a small vestibule, so that one can enter this vestibule and close the door before entering the humidity room. The joints of these doors are well weather-stripped. A corner of the room is shown in Plate 48, A.

The temperature of the room is controlled by a thermograph connected through a pony relay to a bank of lamps fastened overhead and covering an area of about 6 square feet. The lamps remain lighted until the arm of the thermograph records the desired temperature. At this point the indicator of the thermograph makes a contact with a small adjustable platinum arm, thereby closing the circuit from a bell-ringing transformer, which in turn actuates the relay magnet and breaks the light circuit. There is a large tank in the upper part of the room which may be filled with running water and used as a cooler to keep the temperature of the room from going above that desired. At this station, however, it is not ordinarily necessary to use the cooler, as the main laboratory can easily be kept below 70° F., the temperature at which the fiber-testing machine is most used.

The humidity of the room is controlled by an electrical connection through a hydrograph indicator similar to that through the thermograph. When the humidity of the room reaches the desired percentage, as recorded on the hydrograph, the circuit through a $\frac{1}{20}$ h. p. motor which works an atomizer above the tank in the upper part of the room is automatically broken. By means of reducing gears and a crank arm, this motor operates two small air compressers of the bicycle foot-pump type. The two pumps are placed in a horizontal position with their piston rods connected to each other and are also connected through a jointed arm, to the crank pin so that each half turn of the crank causes a forward stroke of one piston and a backward stroke of the other. The air from each pump is conducted to an atomizer in the top of the room. These atomizers are of the household type, but have been modified to fit 1-gallon glass jugs. The greater part of the spray from these atomizers settles into the large water tank, any spray reaching the center of the room being so fine that it is practically all absorbed by the atmosphere before it reaches the floor. The method of pumping is entirely improvised and could easily be replaced by a small electric blower.

Both the thermograph and hydrograph can be quickly set for a new temperature or humidity. The temperature can be regulated with ease at any temperature between 65° and 80° F. and the humidity anywhere

from 35 to 85 per cent. The writer hopes, with certain additions to the room, to make it possible to regulate the humidity at any point between 10 and 90 per cent.

This humidity room has been in constant operation for over seven months, and has proved very satisfactory. It is possible to get a more elaborate equipment and no doubt a more satisfactory one for a larger room, such, for example, as the one in use at the Bureau of Chemistry of the United States Department of Agriculture (*8*), but for a small room and with a comparatively small investment the present arrangement is all that could be desired.

Records of the temperature and the humidity for one week are shown in Plate 48, B. The temperature can easily be regulated at 70° F., with a maximum variation of about 1 degree. The variation in the percentage relative humidity may be regulated to within 2 per cent on the bench where the samples are stored and measured, provided the desired percentage is not over 70. Above this point there is a somewhat larger variation when the door of the humidity room is first opened.

SUMMARY

(1) The breaking-strength determination as a measure of the strength of wool is unsatisfactory because of the wide variations in the size of the individual fibers.

(2) The microscope was found an ineffective means of making a correction for the diameter of the fibers. A micrometer substituted in place of the lower jaw of the testing machine proved to be very efficient in making this correction and reducing the breaking strength to tensile strength or unit stress.

(3) Comparisons of the tensile strengths at five relative humidities—namely, 40, 50, 60, 70, and 80 per cent—showed that the tensile strength of raw wool from four different breeds of sheep decreases as the humidity increases.

(4) Controlled conditions of temperature and humidity were obtained by means of electrical connections through a thermograph and a hydrograph, operating, respectively, a bank of lamps and two atomizers.

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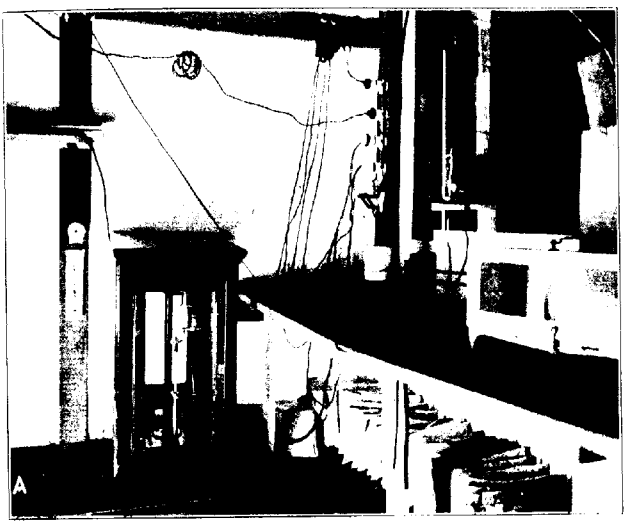
PLATE 48

A—A corner of the humidity room used to test wool fiber.
B—Records of the temperature and humidity during the experiment.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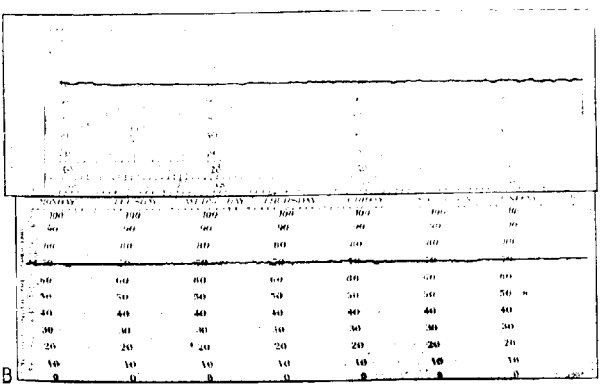
B

AVAILABILITY OF POTASH IN SOME COMMON SOIL-FORMING MINERALS—EFFECT OF LIME UPON POTASH ABSORPTION BY DIFFERENT CROPS

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INTRODUCTION

Little direct information is obtainable regarding the relative availability of potash carried in common soil-forming minerals. The data found are decidedly contradictory. They have either been obtained from the ability of weak solvents to remove potassium or have been adjudged from the resulting optical properties of the minerals after years of subjection to the forces of weathering.

Numerous petrographic analyses of the soils of the United States (*McCaughey and Fry, 1913*) [1] show that only four minerals which carry potash are found in the very fine sand and coarse silt separates. These are biotite, muscovite, orthoclase, and microcline. In many of the residual soils, such as the Porter and Cecil series (*Plummer, 1915*), the micas are found in large quantitites, and must supply much of their potash. Some of the transported soils, such as those of the Atlantic Coastal Plain, carry comparatively little mica, but often are well supplied with microcline and orthoclase.

It has been known for a good many years that certain neutral salts when in contact with the mineral portion of the soil cause an exchange of bases between the salt and soil. Owing to this action, many claims have been made regarding the effects of lime and other compounds for increasing the soluble potash of the inert soil mass. More recent experiments give indications that the effect of lime and gypsum in bringing into solution potash from the mineral portion of the soil is slight or nil. None of these investigations, however, as shown by the following brief review, have thoroughly covered the direct action of lime compounds on those minerals which supply the soil with potassium.

REVIEW OF PREVIOUS INVESTIGATIONS

So far as the writer is aware, Johnstone (*1889*) was the first to report on the stability of micaceous minerals. This investigator found that, after suspension of mica for as much as one year in carbonated water, no alteration could be detected.

Hilgard (*1906, p. 51*), in speaking of soils formed from mica schist, says:

> . . . mica schist, which being a mixture of quartz and mica only, not only weathers very slowly, but also supplies but little of any importance to plants to the soils formed from it.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 314-315.

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Hartwell and Pember (*1908*) conducted experiments with feldspar (variety not given) as the source of potash for plants. Their results led to the conclusion that little could be expected from this material as a source of available potash.

Prianischnikow (*1912*) experimented with a number of crops, using various minerals as the source of potash. From his work conclusions are drawn that biotite and muscovite are superior to feldspar (orthoclase and microcline) as carriers of potash.

Fraps (*1912*) found that all potash is extracted from biotite with strong hydrochloric acid, about one-third from muscovite, and only a small percentage from orthoclase and microcline. Fraps also found that practically no potash is removed from orthoclase and microcline by *N*/5 nitric acid, less than 10 per cent from biotite, and 15 per cent from muscovite.

McCaughey and Fry (*1913*) conclude from observations of the optical properties of the potash-bearing soil-forming minerals that biotite must give up its potash to solution faster than muscovite and orthoclase faster than microcline.

Curry and Smith (*1914*) found from fertilizer experimentation for hay that calcium carbonate and lime have practically no effect on the solubility of soil potash.

Plummer (*1915*) found indications from field experiments that soils with high content of the micas respond less to potash fertilization than do those in which the feldspars predominate.

Clark (*1916, p. 395*) says:

> Muscovite under ordinary circumstances is one of the least alterable of minerals. The feldspar of a granite may be completely kaolinized, while the imbedded plates of mica retain their brilliancy unchanged.

Lyon and Bizzell (*1916*) say, as a result of lysimeter experiments:

> So far as could be ascertained from the potassium in the drainage water and the crop raised on the soil treated with lime and the soil not so treated, there was no liberation of potassium effected by the lime treatment.

Fraps (*1916*) finds only slight gains of potash due to additions of carbonate of lime on the insoluble potash of the soil.

Briggs and Breazeale (*1917*) find that calcium-hydrate solutions do not modify the solubility of potash in orthoclase or orthoclase-bearing soils.

In view of the variance of results set forth in the foregoing discussions, it would seem desirable that experimentation be carried out to determine the relative availability of potash in the minerals which supply the soil with this constituent.

EXPERIMENTAL WORK

The minerals used were as representative and free from impurities as could be obtained. Each specimen was ground to an impalpable powder and sifted through the finest grade of bolting cloth.

Table I gives the composition of the materials used in this work.

TABLE I.—*Composition of materials used*

Material.	Nitrogen (N).	Available phosphoric acid (P_2O_5).	Potash (K_2O).	Lime (CaO).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Dried blood	13.70	.....	.....	.....
Acid phosphate	.....	16.20	.....	(a)
Potassium sulphate	.....	.....	50.80	.....
Biotite	.....	.....	8.45	(a)
Muscovite	.....	.....	9.14	(a)
Orthoclase	.....	.....	13.40	(a)
Microcline	.....	.....	14.40	(a)
Precipitated calcium carbonate	.....	.....	.....	56.02

a Not determined.

The potash contents, which are very close to the theoretical for the individual minerals, indicate specimens of exceptional purity. Petrographic examinations of the feldspars give all characteristic optical properties of pure orthoclase and microcline, respectively.

SOLUBILITY OF MINERAL POTASH IN CARBONATED WATER AND THE EFFECT OF CALCIUM BICARBONATE THEREON

Water charged with carbon dioxid is generally considered the chief solvent of inert plant nutrients of the soil. To obtain the true availability of any dormant constituent, several extractions are necessary—that is, until a point is reached at which no appreciable amount goes into solution.

Calcium bicarbonate results from the presence of any basic calcium compounds in the soil, and is the form of lime which naturally functions in the exchange of bases.

For comparison with soil conditions, carbonated water and calcium bicarbonate were selected for measuring the availabilty of potash in the four soil-forming minerals.

Distilled water was saturated under pressure with carbon dioxid. The solution of calcium bicarbonate [$Ca(HCO_3)_2$] was $N/20$ in strength, and contained an excess of carbon dioxid to prevent the precipitation of calcium carbonate ($CaCO_3$). Thirty gm. of each material and 200 cc. of the solvent were placed in 500-cc. flasks and agitated in an end-over-end slaking machine for 96 hours. At the end of this time suspended matter was allowed to settle, the solutions were clarified, and potash was determined colormetrically, according to methods given by Schreiner and Failyer (*1906*). The residue was thrown on a filter and washed free of potash, after which it was again extracted as before, and the process repeated four times.

The results obtained will be found in Table II.

TABLE II.—*Solubility of potash in common soil-forming minerals, with the effect of calcium bicarbonate*

[Results expressed as parts per million of potassium oxid]

Mineral.	Amount taken.		Extractions with distilled water.						Extractions with carbonated water.						Extractions with calcium bicarbonate in carbonated water.						Gain or loss for calcium bicarbonate.
			1	2	3	4	5	Total.	1	2	3	4	5	Total.	1	2	3	4	5	Total.	
	Gm.	*Cc.*																			
Biotite	30	200	11.0	6.1	5.6	1.2	1.0	24.9	116.0	87.0	49.6	16.0	5.6	274.2	108.0	92.0	44.0	10.0	6.4	251.4	—22.8
Do	30	200	12.6	6.0	5.0	3.1	.6	27.3	109.0	84.0	45.7	11.4	8.2	258.3	110.0	80.0	41.0	15.0	7.9	253.9	— 4.4
Do	30	200	10.0	7.9	5.4	1.0	.6	24.9	111.0	80.0	40.2	14.3	6.8	252.3	108.0	85.0	42.0	13.4	7.0	255.4	+ 3.1
Do	30	200	10.0	8.0	6.2	4.0	.2	28.9	108.0	89.0	43.0	13.6	6.6	260.2	114.0	90.0	46.0	14.0	5.8	269.2	+ 9.0
Average			10.9	7.0	5.5	2.3	.6	26.3	111.0	85.0	44.6	13.8	6.8	261.3	110.0	86.8	43.3	13.1	6.6	257.5	— 3.8
Muscovite	30	200	13.6	6.0	4.0	2.4	.4	26.4	89.4	40.0	26.0	11.1	4.0	170.5	92.0	43.0	24.0	12.0	3.9	174.9	+ 4.4
Do	30	200	11.4	5.0	3.8	1.8	.3	22.3	88.0	41.2	24.7	12.6	2.0	168.5	90.0	39.0	23.0	10.9	3.2	166.1	— 2.4
Do	30	200	10.2	5.4	6.2	.8	.7	23.3	89.0	41.0	24.7	13.6	3.2	170.9	86.0	46.0	27.0	13.0	2.4	174.4	+ 3.5
Do	30	200	10.0	6.0	6.0	1.2	1.8	25.0	86.0	41.0	26.3	12.0	2.4	167.8	86.0	40.0	25.0	11.8	3.9	166.7	— 1.1
Average			11.3	5.6	5.0	1.5	.8	24.2	88.1	40.8	25.4	12.2	2.9	169.4	88.5	42.0	24.8	11.8	3.5	170.5	+ 1.1
Orthoclase	30	200	10.6	6.6	4.0	2.0	.6	23.8	41.8	21.4	17.6	12.4	4.6	97.8	44.0	20.0	18.6	14.2	5.0	101.8	+ 4.0
Do	30	200	8.9	5.4	3.8	1.6	.4	20.1	37.3	21.0	16.0	11.7	5.6	91.6	40.0	21.6	15.0	10.0	4.2	90.8	— .8
Do	30	200	9.4	5.6	2.6	.6	.3	18.4	35.3	23.0	17.6	11.7	5.0	92.6	39.0	26.0	16.0	10.0	6.4	97.7	+ 4.8
Do	30	200	9.4	5.0	3.0	1.2	.6	19.2	.....	.....	.....	.....	.....	.....	33.0	23.0	12.0	12.0	6.4	[a]86.4	.....
Average			9.7	5.7	3.4	1.4	.5	20.4	38.1	21.4	17.1	11.9	5.1	94.0	39.0	22.6	15.4	11.3	5.5	96.7	+ 2.7
Microcline	30	200	10.8	6.0	2.0	.8	1.0	19.8	30.6	16.0	7.9	6.6	3.0	64.1	28.0	16.6	8.4	5.8	3.8	62.6	— 1.5
Do	30	200	11.3	4.1	2.4	.6	.4	18.8	25.6	17.6	8.2	6.0	4.8	62.2	30.0	19.4	7.0	6.1	5.3	67.8	+ 5.6
Do	30	200	8.0	5.0	1.6	1.2	.2	16.0	26.6	15.8	8.2	5.6	5.0	61.2	25.4	15.0	8.0	5.6	4.6	58.6	— 2.6
Do	30	200	10.0	5.0	2.0	.9	.2	18.0	23.3	17.6	8.0	6.4	2.8	58.1	22.0	14.8	7.6	6.6	5.8	54.8	— 3.2
Average			9.8	5.0	2.0	.9	.4	18.1	26.5	16.8	8.1	6.1	3.9	61.4	26.4	16.4	7.8	6.0	4.4	60.9	— .4

[a] Not included in average.

The results obtained from these experiments indicate only small differences in the solubility of potash in distilled water. Biotite and muscovite appear to give up somewhat more of this plant nutrient to water than do the feldspars. These differences are small, however, and may be due to experimental error.

With carbonic acid as the solvent the divergence in the amounts of potash going into solution is more marked. More than four times as much potash of biotite is dissolved as is carried by microcline. Muscovite stands next to biotote in the solubility of its potash, and orthoclase is slightly ahead of microcline.

These findings agree rather closely with the vegetative experiments detailed later and follow the same order as those of Fraps (*1912*), in which a weak solution of nitric acid was used as the solvent.

Calcium bicarbonate has not shown any power to unlock potash from any of the minerals. With biotite and microcline there are slight losses of potash when this material is used in connection with water charged with carbon dioxid. Only very small gains from the use of the bicarbonate are discernible with muscovite and orthoclase, gains so small as to be considered negligible and to have no practical significance.

Briggs and Breazeale (*1917*) have recently reached the same conclusions from the use of calcium hydroxid and gypsum on orthoclase and certain orthoclase-bearing soils.

VEGETATIVE EXPERIMENTS WITH THE COMMON SOIL-FORMING MINERALS

The solubility investigations just given have shown rather marked differences in the power with which potash is held in the two micas and feldspars. For the purpose of supplementing the laboratory data, pot experiments were begun in which four different crops were grown out of doors to maturity. These were oats (*Avena sativa*), soybeans (*Soja max*), rye (*Secale cereale*), and cowpeas (*Vigna sinensis*).

Description of Pot Experiments

SOIL USED

The soil used in this investigation was taken from the no-treatment plots of the Edgecombe (N. C.) Branch Station, where experiments to determine its fertilizer requirements have been running for the past 15 years. The field tests (*Kilgore et al., 1914*) indicate rather conclusively that potash is one of the limiting elements of this soil; also that the available plant nutrients have been reduced to a minimum on the plots receiving no additions. The soil was taken from the plots to a depth of 6⅔ inches. Tables III and IV give the chemical and mineralogical composition of the soil used.

TABLE III.—*Chemical composition of soil used*

Plant nutrient.	Percentage composition of oven-dried soil.	Quantity per acre of 2,000,000 pounds of soil.
		Pounds.
Nitrogen	0.032	640
Phosphoric acid	.026	520
Potash	.094	1,880
Soda	.041	820
Lime	.154	3,080
Magnesia	.082	1,640

TABLE IV.—*Petrographic analysis of soil*

Percentage of minerals not quartz in—		Abundant minerals not quartz in—		Less abundant minerals not quartz in—		Remarks.
Sand.	Silt.	Sand.	Silt.	Sand.	Silt.	
2-4	5-8....	None..	None..	Orthoclase (residues), microcline, epidote, tourmaline, magnetite, hornblende.	Epidote, tourmaline, zircon, rutile, magnetite, sillimanite, hornblende, muscovite, biotite, garnet.	Soil characterized by low content of minerals other than quartz. Only trace of mica present. Minerals existing are of a refractory nature.

CONDITIONS OF PLANT GROWTH

The equivalent of 40 pounds of oven-dried soil was carefully weighed out, the various plant nutrients were added in the amounts given in Table V and were mixed thoroughly by rolling over and over on canvas cloth. This was transferred to 4-gallon glazed earthenware pots. Sufficient drainage was obtained through small openings on the lower side of each pot.

Nitrogen and phosphoric acid were added to all pots two weeks before seeding each crop. Potash and lime were added only at the beginning of the experiment.

The rates of application were made on the basis of 200 and 400 pounds of potash per acre. For convenience of expressing the data obtained, in Tables V to X the treatments are referred to as the mineral which carries potash in weights of 200 pounds per acre. The figure "2" before the name of the potash carrier indicates that this plant nutrient has been applied at the rate of 400 pounds per acre.

This work was conducted out of doors in a cage of ¼-inch-mesh poultry wire. Excessive heat is prevented in summer by a lattice-work cover similar to those used in covering ginseng beds. During spring and summer the pots were placed on benches 2 feet above the surface of the ground. In winter they were buried in a mixture of sawdust and soil sufficiently deep to prevent freezing.

TABLE V.—*Rate of application of plant nutrients*

Carrier.	Quantity of carrier per pot.	Quantity of plant nutrients (pounds per acre of 2,000,000 pounds of soil).			
		Nitrogen.	Phosphoric acid.	Potash.	Lime.
	Grams.				
Dried blood	4.834	73	.....	.....	.....
Acid phosphate	13.424	.....	224	.....	(a)
Potassium sulphate	3.570	.....	.....	200	.....
2 potassium sulphate	7.140	.....	.....	400	.....
Biotite	21.488	.....	.....	200	(a)
2 biotite	42.976	.....	.....	400	(a)
Muscovite	19.872	.....	.....	200	(a)
2 muscovite	39.744	.....	.....	400	(a)
Orthoclase	13.548	.....	.....	200	(a)
2 orthoclase	27.096	.....	.....	400	(a)
Microcline	12.552	.....	.....	200	(a)
2 microcline	25.104	.....	.....	400	(a)
Precipitated calcium carbonate	33.420	.....	.....	.....	2,000
2 precipitated calcium carbonate	66.840	.....	.....	.....	4,000

a Not determined.

Enough water was added each day, when necessary, to keep the soil well moistened during periods of plant growth.

Acid-washed quartz was placed over each pot after seeding to act as a mulch.

OAT CROPS

On March 24, 1916, 20 seeds of the Burt variety of oats were planted to each pot. After germination the plantlets were drawn down to a uniform stand of 12 per pot. On the following June 10 the oat crop was harvested after reaching maturity. Owing to the inability of removing all the roots, only the portion of the plants above ground was considered.

Potash was determined separately in the grain and straw of each pot. Table VI contains the data obtained.

TABLE VI.—*Weight of oat crop and potash removed from soil*

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Gain over no potash.
Potassium sulphate	13.8	24.9	38.7	37.0	82.4	0.065	0.306	0.371	0.363	0.305
Do	16.4	22.4	38.8			.083	.300	.383		
Do	12.7	20.9	33.7			.062	.284	.346		
2 potassium sulphate	17.2	27.6	44.8	44.8	99.8	.086	.366	.452	.454	.396
Do	18.0	24.9	42.9			.088	.366	.454		
Do	20.0	26.8	46.8			.092	.367	.457		
2 potassium sulphate plus calcium carbonate	21.1	28.0	49.1	44.8	99.8	.107	.434	.541	.482	.423
Do	20.0	25.0	45.0			.092	.425	.417		
Do	17.6	22.8	40.4			.088	.400	.488		

TABLE VI.—*Weight of oat crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Gain over no potash.
2 potassium sulphate plus 2 calcium carbonate	18.6	24.0	42.6	44.9	100.0	0.102	0.410	0.512	0.474	0.409
Do	19.4	26.0	45.4			.087	.422	.409		
Do	20.9	25.8	46.7			.084	.416	.502		
Biotite	9.9	15.0	24.9	28.7	63.9	.039	.234	.273	.307	.244
Do	10.6	18.6	29.2			.053	.264	.321		
Do	12.4	19.8	32.2			.056	.271	.327		
2 biotite	11.2	17.5	28.7	30.0	66.8	.058	.241	.299	.299	.234
Do	13.0	20.6	33.6			.062	.262	.324		
Do	10.8	16.9	27.7			.040	.236	.276		
2 biotite plus calcium carbonate	9.8	17.8	27.6	31.0	69.0	.040	.240	.280	.299	.234
Do	12.6	20.9	33.5			.052	.256	.308		
Do	10.6	21.4	32.0			.040	.262	.308		
2 biotite plus 2 calcium carbonate	10.6	16.4	27.0	29.1	64.8	.044	.228	.272	.278	.213
Do	13.2	18.9	32.1			.050	.240	.290		
Do	13.0	17.0	30.0			.052	.219	.271		
Muscovite	6.4	14.4	20.8	21.3	47.4	.029	.218	.247	.303	.238
Do	6.4	14.0	20.4			.029	.200	.229		
Do	7.9	15.9	23.8			.031	.224	.255		
2 muscovite	7.0	16.0	23.0	23.8	53.0	.033	.206	.239	.241	.179
Do	7.8	16.9	24.7			.036	.200	.236		
Do	8.0	15.8	23.8			.040	.218	.258		
2 muscovite plus calcium carbonate	6.8	13.6	20.4	22.3	49.6	.030	.196	.226	.244	.179
Do	7.1	16.0	23.1			.032	.218	.250		
Do	7.3	16.0	23.3			.033	.224	.257		
2 muscovite plus 2 calcium carbonate	7.0	15.6	22.6	21.3	47.4	.029	.230	.259	.240	.175
Do	6.9	13.8	20.7			.026	.206	.232		
Do	7.6	13.0	20.6			.031	.199	.230		
Orthoclase	4.2	9.4	13.6	12.3	27.3	.018	.141	.159	.143	.075
Do	3.6	8.6	12.2			.016	.128	.144		
Do	3.6	7.5	11.1			.016	.110	.126		
2 orthoclase	3.8	10.9	14.7	14.3	31.8	.012	.162	.174	.172	.107
Do	4.8	10.8	15.6			.019	.160	.179		
Do	3.0	9.8	12.8			.018	.146	.164		
Do	4.1	9.0	13.1	12.1	26.9	.020	.154	.174	.148	.073
Do	2.9	7.8	10.7			.010	.126	.136		
Do	3.8	8.6	12.4			.016	.120	.136		
2 orthoclase plus calcium carbonate	3.9	8.6	12.5	13.7	30.7	.018	.118	.136	.165	.100
Do	3.0	10.0	13.0			.018	.156	.174		
Do	4.6	11.1	15.7			.022	.164	.186		
Microcline	1.8	6.2	8.0	6.4	14.2	.009	.083	.092	.081	.016
Do	.8	5.0	5.8			.004	.076	.080		
Do	1.0	4.4	5.4			.007	.069	.077		
2 microcline	2.0	5.6	7.6	6.9	15.4	.008	.068	.076	.075	.010
Do	1.2	6.6	7.8			.006	.084	.090		
Do	.6	4.8	5.4			.003	.057	.060		
2 microcline plus calcium carbonate	1.1	5.0	6.1	7.1	15.8	.004	.072	.076	.080	.015
Do	2.4	7.0	9.4			.007	.088	.095		
Do	.9	4.9	5.8			.002	.069	.071		
2 microcline plus 2 calcium carbonate	.5	4.5	5.0	6.3	14.0	.001	.064	.065	.077	.012
Do	1.7	4.2	5.9			.003	.074	.077		
Do	2.2	5.8	8.0			.006	.083	.089		
Control (no potash)	1.6	4.0	5.6	5.1	11.3	.002	.072	.074	.065	.....
Do	.5	3.8	4.3			.003	.051	.054		
Do	.5	4.9	5.4			.004	.063	.067		
Control (no potash) plus calcium carbonate	.9	5.0	5.9	4.8	10.7	.002	.072	.044	.058	None.
Do	.8	3.2	4.0			.002	.056	.058		
Do	1.6	2.8	4.4			.003	.041	.074		
Control (no potash) plus 2 calcium carbonate	1.1	4.0	5.1	4.3	9.5	.002	.066	.068	.062	None.
Do	.9	3.8	4.7			.004	.058	.062		
Do	.5	2.8	3.3			.003	.053	.056		

These data show quite conclusively that the oat plant is capable of extracting potash from the soil minerals at different rates. When the greatest yield, 2 potassium sulphate and 2 calcium carbonate, is given the rank of 100, the following order of plant growth is obtained: Two biotite plus 2 calcium carbonate reaches 69; 2 muscovite alone 53; 2 orthoclase alone 31.8; 2 microcline plus calcium carbonate 15.8; and no potash without calcium carbonate 11.3.

This soil responds markedly to potash fertilization, as shown by the oat yields. Soluble potash produces growth to the extent of 44.8 gm. per pot; where no potash material is applied only 5.1 gm. per pot is secured.

To judge from the plant growth, lime has not made available any of the insoluble potash applied in the form of minerals. In many instances the yield has been slightly reduced where the carbonate has been used.

The results of plant growth fertilized with double applications of potash and lime are shown graphically in figure 1.

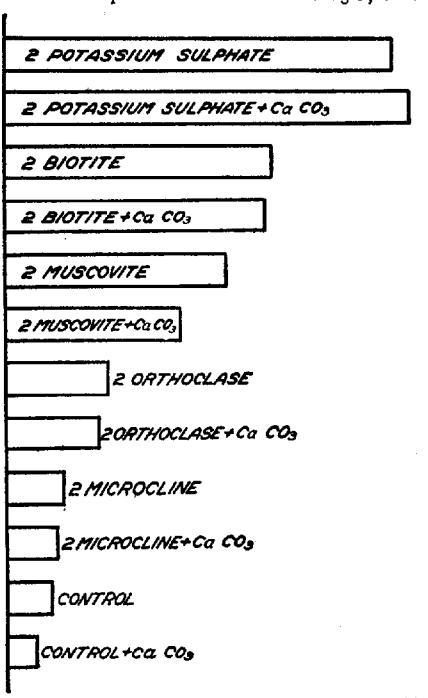

FIG. 1.—Rate of growth of oats under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Considerably more potash has been recovered in the crop when applied in the soluble form. The order of potash recovery follows the same order as crop yield, biotite showing the greatest and microcline the least. Lime has not increased this recovery in any of the treatments (Pl. 49, A).

SOYBEAN CROP

After harvesting the oats the quartz mulch was removed; the roots of the oats were finely ground and mixed thoroughly with the soil.

All pots were inoculated with as nearly the same number of a pure culture of *Bacillus radicicola* as could be done.

On June 19, 10 seeds of Mammoth Yellow variety of soybean were seeded to each pot. These were drawn down after germination to a

uniform stand of five plantlets per pot. The crop was harvested on September 25, after maturing seed.

In Table VII will be found the results obtained.

TABLE VII.—*Weight of soybean crop and potash removed from soil*

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Seed.	Hay.	Total.	Average total.	Relative rank of average.	Seed.	Hay.	Total.	Average total.	Gain over no potash.
Potassium sulphate	14.8	63.4	78.2	77.3	63.0	0.160	0.382	0.542	0.545	0.409
Do	14.1	60.9	75.0			.150	.376	.526		
Do	15.7	63.0	78.7			.174	.392	.566		
2 potassium sulphate	15.2	68.0	83.2	85.5	61.5	.271	.526	.797	.802	.666
Do	16.9	70.0	86.9			.280	.531	.811		
Do	16.6	70.0	86.6			.279	.520	.799		
2 potassium sulphate plus calcium carbonate	27.7	82.2	109.9	112.8	90.8	.526	.556	1.082	1.099	.963
Do	26.0	86.3	112.3			.482	.562	1.044		
Do	28.2	88.0	116.2			.566	.604	1.170		
2 potassium sulphate plus 2 calcium carbonate	29.8	91.0	120.8	124.2	100.0	.574	.622	1.196	1.234	1.098
Do	33.2	93.0	126.2			.642	.650	1.292		
Do	32.1	93.6	125.7			.569	.646	1.215		
Biotite	9.8	56.1	65.7	66.1	53.2	.167	.403	.570	.567	.431
Do	8.7	55.0	63.7			.152	.386	.548		
Do	10.6	57.9	68.5			.170	.414	.584		
2 biotite	10.3	55.0	65.0	64.8	52.1	.270	.400	.570	.575	.339
Do	9.0	52.0	61.0			.158	.377	.534		
Do	11.4	56.6	68.0			.204	.416	.620		
2 biotite plus calcium carbonate	17.8	70.3	88.1	88.3	71.1	.307	.490	.797	.791	.655
Do	16.5	68.6	85.1			.286	.462	.748		
Do	18.4	72.4	91.8			.324	.504	.828		
2 biotite plus 2 calcium carbonate	16.0	68.2	84.2	87.8	70.7	.314	.440	.754	.789	.653
Do	17.4	71.8	89.2			.306	.468	.774		
Do	17.0	73.0	90.0			.310	.530	.840		
Muscovite	9.4	50.2	59.6	56.8	45.7	.162	.364	.526	.528	.392
Do	10.8	51.7	52.5			.171	.360	.531		
Do	10.0	48.3	58.3			.170	.358	.528		
2 muscovite	11.6	47.6	59.2	58.7	47.2	.180	.351	.531	.534	.388
Do	10.8	46.9	57.7			.172	.350	.522		
Do	10.4	50.2	60.6			.175	.376	.551		
2 muscovite plus calcium carbonate	13.8	58.0	71.8	73.9	59.5	.234	.418	.652	.663	.527
Do	12.2	60.0	72.2			.226	.420	.646		
Do	14.9	63.0	77.9			.245	.446	.691		
2 muscovite plus 2 calcium carbonate	11.9	57.0	68.9	69.7	56.1	.189	.400	.589	.625	.489
Do	13.6	59.8	73.4			.246	.412	.658		
Do	12.0	56.4	66.4			.225	.402	.627		
Orthoclase	6.0	30.6	36.6	36.5	29.2	.112	.281	.393	.388	.252
Do	5.6	34.0	39.6			.106	.294	.400		
Do	4.8	28.6	33.4			.100	.272	.372		
2 orthoclase	5.0	31.0	36.0	40.4	32.5	.096	.290	.386	.408	.272
Do	6.6	36.2	42.8			.116	.306	.422		
Do	6.3	36.2	42.5			.114	.304	.418		
2 orthoclase plus calcium carbonate	9.1	49.2	58.3	55.3	44.5	.133	.344	.477	.473	.337
Do	8.6	46.0	54.6			.130	.346	.476		
Do	7.9	45.1	53.0			.135	.232	.467		
2 orthoclase plus 2 calcium carbonate	7.6	45.3	52.9	55.6	44.7	.132	.330	.462	.468	.332
Do	8.4	47.0	55.4			.130	.338	.468		
Do	9.2	49.4	58.6			.129	.346	.475		
Microcline	3.2	16.1	19.3	18.0	14.4	.066	.112	.178	.166	.030
Do	2.6	15.6	18.2			.052	.106	.158		
Do	2.6	14.2	16.8			.050	.111	.161		
2 microcline	3.4	15.0	18.4	17.9	14.4	.069	.105	.174	.166	.030
Do	2.8	13.8	16.6			.048	.100	.148		
Do	4.1	14.6	18.7			.072	.103	.175		
2 microcline plus calcium carbonate	5.3	19.1	24.4	25.8	20.7	.078	.114	.192	.197	.061
Do	6.0	20.7	26.7			.080	.119	.199		
Do	5.8	20.5	26.3			.076	.120	.196		
2 microcline plus 2 calcium carbonate	5.4	21.6	27.0	26.5	21.3	.080	.122	.202	.197	.061
Do	6.3	20.0	26.3			.076	.119	.195		
Do	6.3	22.0	26.3			.075	.119	.194		

TABLE VII.—*Weight of soybean crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Seed.	Hay.	Total.	Average total.	Relative rank of average.	Seed.	Hay.	Total.	Average total.	Gain over no potash.
Control (no potash)	2.0	11.0	13.0	13.6	10.9	0.046	0.086	0.132	0.136	
Do	2.0	10.8	12.8			.053	.072	.125		
Do	3.6	12.6	15.2			.062	.091	.153		
Control (no potash) plus calcium carbonate	5.0	17.6	22.6	21.6	17.3	.065	.100	.165	.161	0.025
Do	4.6	16.0	20.6			.058	.096	.154		
Do	4.0	16.6	20.6			.062	.102	.163		
Control (no potash) plus 2 calcium carbonate	4.2	16.0	20.2	21.8	17.5	.062	.102	.164	.166	.030
Do	5.6	18.3	23.9			.069	.106	.175		
Do	4.0	17.4	21.5			.060	.099	.159		

The results shown in Table VII follow the same order regarding the relative availability of the insoluble potashas with the oat crop. Greatest growth has been obtained from biotite, then muscovite, orthoclase, and the least from microcline.

Calcium carbonate has materially increased plant growth and the potash recovered in the crop when supplied with potassium sulphate, biotite, and muscovite; but to a much lesser extent with the feldspar and where no potash was added. This should not be taken to indicate that lime has been exchanged directly for potash in the applied minerals, but has produced conditions in the soil more favorable to the growth of the legume. In this way hardier plants are produced which are capable of extracting more of this constituent from the minerals in which it is not so securely held.

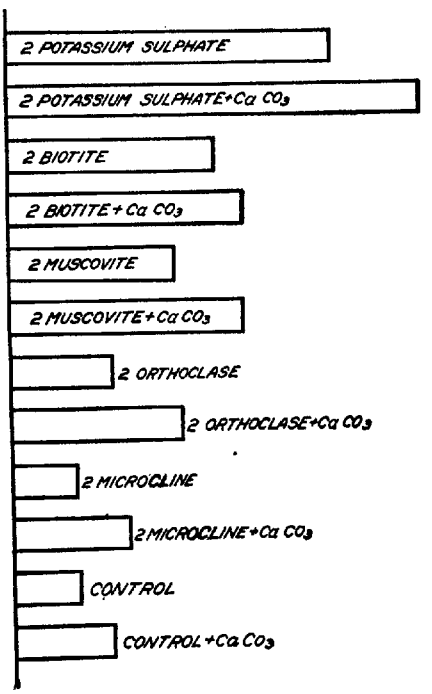

FIG. 2.—Rate of growth of soybeans under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Figure 2 shows graphically the rates of the growth of soybeans fertilized with double applications of potash and lime. (See also Pl. 49, B.)

RYE CROP

The soybean roots were ground as those of the oats and thoroughly incorporated with the soil. On October 3, 1916, 20 rye seed were added to each pot, of which 12 plantlets were left to mature. On June 4, 1917, the rye was harvested. Table VIII contains the data of this harvest.

TABLE VIII.—*Weight of rye crop and potash removed from soil*

Treatment.	Dry matter (grams).					Potash removed (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Average gain over no potash.
Potassium sulphate	8.1	29.1	37.2			0.046	0.162	0.208		
Do	7.5	28.0	35.5	36.5	76.5	.040	.151	.191	0.202	0.129
Do	8.8	28.0	36.8			.048	.158	.206		
2 potassium sulphate	10.4	29.9	40.3			.065	.260	.325		
Do	9.6	28.4	38.0	38.5	80.7	.051	.255	.306	.312	.247
Do	9.6	27.6	37.2			.054	.250	.304		
2 potassium sulphate plus calcium carbonate	12.0	30.0	42.0			.069	.271	.340		
Do	14.0	34.0	48.0	46.5	97.4	.074	.274	.358	.349	.276
Do	13.6	36.0	49.6			.076	.275	.351		
2 potassium sulphate plus 2 calcium carbonate	12.8	32.6	45.4			.070	.268	.338		
Do	13.4	37.4	50.8	47.7	100.0	.072	.280	.352	.353	.280
Do	14.4	38.2	52.6			.086	.282	.368		
Biotite	6.4	26.6	33.0			.039	.186	.225		
Do	5.8	26.0	31.0	32.0	67.1	.036	.180	.216	.219	.146
Do	6.0	25.2	31.2			.042	.174	.216		
2 biotite	7.0	27.2	34.2			.040	.194	.234		
Do	6.4	25.0	31.4	32.3	67.7	.039	.174	.213	.222	.149
Do	6.9	24.6	31.5			.044	.175	.219		
2 biotite plus calcium carbonate	6.7	26.0	32.7			.044	.180	.224		
Do	6.6	26.0	32.6	32.0	67.1	.040	.180	.220	.225	.152
Do	5.4	25.4	30.8			.038	.173	.211		
2 biotite plus 2 calcium carbonate	7.2	27.0	34.2			.048	184	.232		
Do	5.7	26.0	31.7	32.2	67.5	.036	.180	.216	.218	.145
Do	5.7	24.9	30.6			.033	.172	.205		
Microcline	2.0	10.4	12.4			.016	.078	.094		
Do	1.6	11.6	13.2	11.7	24.5	.014	.083	.107	.093	.020
Do	.8	8.8	9.6			.009	.069	.078		
2 microcline	1.4	10.0	11.4			.009	.074	.083		
Do	.9	8.8	9.7	9.9	20.7	.007	.062	.069	.071	None.
Do	.9	7.6	8.5			.006	.056	.062		
2 microcline plus calcium carbonate	1.8	8.8	10.6			.008	.066	.074		
Do	.5	7.0	7.5	9.3	19.4	.003	.073	.076	.078	.005
Do	.6	9.2	9.8			.004	.080	.084		
2 microline plus 2 calcium carbonate	1.0	8.8	9.8			.005	.064	.069		
Do	.5	9.0	9.5	9.9	20.7	.002	.069	.071	.073	None.
Do	.9	9.6	10.5			.004	.074	.074		
Control (no potash)	1.6	8.4	10.0			.007	.068	.075		
Do	1.6	8.0	9.6	9.7	20.5	.007	.064	.071	.073	.....
Do	.3	7.1	7.4			.001	.071	.072		
Control (no potash) plus calcium carbonate	.3	9.0	9.3			.001	.066	.067		
Do	.6	8.0	8.6	9.3	19.4	.003	.060	.063	.066	None.
Do	1.4	8.6	10.0			.007	.062	.069		
Control (no potash) plus 2 calcium carbonate	1.4	7.6	9.0			.007	.064	.071		
Do	1.4	6.9	8.3	8.8	18.4	.006	.063	.069	.071	Do.
Do	.7	8.4	9.1			.004	.069	.073		
Muscovite	6.0	22.0	28.0			.041	.154	.195		
Do	4.8	20.4	25.2	27.3	57.2	.039	.143	.182	.192	.119
Do	5.1	23.7	28.8			.040	.160	.200		
2 muscovite	5.0	20.0	25.0			.046	.140	.186		
Do	4.4	24.0	28.4	27.1	56.8	.044	.158	.202	.197	.124
Do	5.2	22.6	27.8			.048	.156	.204		
2 muscovite plus calcium carbonate	6.3	21.6	27.9			.050	.153	.203		
Do	5.2	22.4	27.6	26.8	56.1	.040	.146	.186	.193	.120
Do	4.9	20.8	25.7			.052	.140	.192		
2 muscovite plus 2 calcium carbonate	4.8	20.8	25.6			.043	.140	.183		
Do	6.1	22.4	28.5	27.5	57.6	.052	.156	.208	.198	.125
Do	5.8	22.6	28.4			.050	.154	.204		

TABLE VIII.—*Weight of rye crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).					Potash removed (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Average gain over no potash.
Orthoclase	3.0	15.6	18.6			0.022	0.106	0.128		
Do	3.8	15.0	18.8	18.5	38.7	.024	.100	.124	0.126	0.053
Do	3.1	15.2	18.3			.025	.102	.127		
2 orthoclase	2.8	14.6	17.4			.020	.100	.120		
Do	2.8	13.8	16.6	17.8	37.3	.020	.090	.110	.122	.049
Do	3.6	15.8	19.4			.026	.110	.136		
2 orthoclase plus calcium carbonate	3.6	16.0	19.6			.024	.102	.126		
Do	4.0	17.0	21.0	19.5	40.8	.029	.103	.132	.126	.053
Do	2.1	15.8	17.9			.020	.100	.120		
2 orthoclase plus 2 calcium carbonate	3.00	16.6	19.6			.025	.104	.129		
Do	2.6	16.4	19.0	19.0	39.8	.019	.104	.123	.126	.053
Do	2.4	16.0	18.4			.023	.103	.126		

Rye seems to remove more potash from the micas than from the feldspars. Microcline gives practically the same yield as when no potash is added. Orthoclase has given slightly greater yields, but nothing like so great as those of biotite and muscovite (Pl. 49, C).

In figure 3 will be found the graphical representation of rye produced with double applications of potash and lime.

As with the crop of oats, lime has produced very slight, if any, effect on liberating potash from the insoluble forms. Neither has crop growth nor the amount of potash removed by the crop been increased by its use

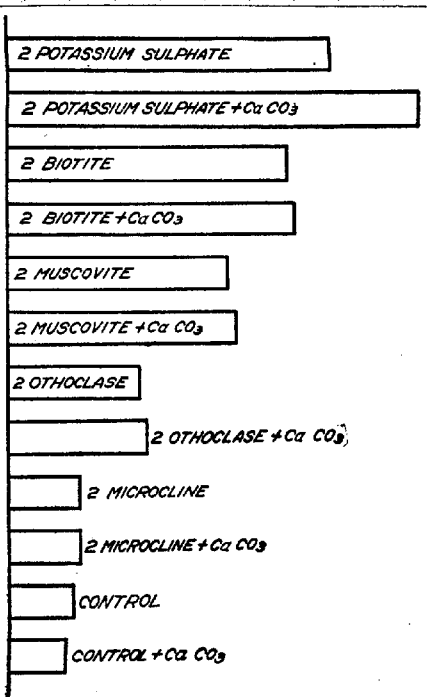

Fig. 3.—Rate of growth of rye under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Cowpea Crop

The rye roots were ground and mixed with the soil as with the preceding crops, and pots seeded with 15 seeds of Manetta variety of cowpeas on June 7, 1917. After germination, plantlets were reduced to six per pot. The crop was harvested on the following September 17, before the cowpeas had matured. This was done, as the plants had been injured by mildew.

In Table IX will be found the results of this experiment.

Table IX.—*Weight of cowpea crop and potash removed from soil*

Treatment.	Dry matter (grams).			Potash removed (grams).		
	Whole plant.	Average total.	Relative rank of average.	Whole plant.	Average total.	Average gain over no potash.
Potassium sulphate	30.4	27.8	63.3	0.374	0.356	0.272
Do	27.0			.350		
Do	26.1			.346		
2 potassium sulphate	31.0	31.5	71.7	.465	.469	.385
Do	33.0			.476		
Do	30.6			.468		
2 potassium sulphate plus calcium carbonate	40.2	43.8	99.7	.643	.661	.577
Do	44.6			.669		
Do	46.7			.672		
2 potassium sulphate plus 2 calcium carbonate	48.0	43.9	100.0	.720	.698	.612
Do	42.0			.694		
Do	41.6			.680		
Biotite	20.0	19.1	43.5	.300	.313	.229
Do	17.6			.326		
Do	19.7			.314		
2 biotite	21.4	20.3	46.2	.306	.306	.222
Do	20.6			.312		
Do	18.3			.300		
2 biotite plus calcium carbonate	24.0	24.9	56.7	.374	.376	.292
Do	26.0			.386		
Do	24.8			.370		
2 biotite plus 2 calcium carbonate	23.0	24.0	54.6	.302	.303	.219
Do	22.0			.294		
Do	27.0			.312		
Muscovite	14.0	15.0	34.1	.224	.228	.144
Do	16.8			.236		
Do	14.3			.224		
2 muscovite	15.0	14.3	32.5	.270	.263	.179
Do	15.0			.264		
Do	13.0			.256		
2 muscovite plus calcium carbonate	20.0	21.5	48.9	.302	.310	.226
Do	22.6			.310		
Do	22.0			.318		
2 muscovite plus 2 calcium carbonate	25.0	24.1	54.8	.326	.318	.234
Do	23.0			.316		
Do	24.2			.314		
Orthoclase	10.0	8.1	18.4	.150	.138	.054
Do	8.4			.126		
Do	8.0			.130		

TABLE IX.—*Weight of cowpea crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).			Potash removed (grams).		
	Whole plant.	Average total.	Relative rank of average.	Whole plant.	Average total.	Average gain over no potash.
2 othoclase	9.6			0.153		
Do	11.2	10.1	23.0	.160	0.152	0.068
Do	9.4			.142		
2 orthclase plus calcium carbonate	14.6			.202		
Do	16.6	16.1	36.4	.174	.195	.111
Do	17.8			.210		
2 orthoclase plus 2 calcium carbonate	15.0			.222		
Do	16.0	16.3	36.5	.197	.201	.117
Do	17.9			.184		
Microcline	4.8			.081		
Do	5.6	5.5	12.4	.091	.088	.004
Do	6.2			.094		
2 microcline	4.0			.068		
Do	4.0	4.6	10.5	.084	.078	None.
Do	5.9			.082		
2 microcline plus calcium carbonate	9.4			.108		
Do	10.2	9.4	21.4	.110	.105	.021
Do	8.6			.096		
2 microcline plus 2 calcium carbonate	10.0			.113		
Do	10.2	9.6	21.8	.114	.106	.022
Do	8.6			.092		
Control (no potash)	5.0			.090		
Do	4.8	4.6	10.5	.084	.084	.....
Do	4.0			.078		
Control (no potash) plus calcium carbonate	10.0			.108		
Do	7.2	8.5	19.3	.102	.105	.021
Do	8.3			.105		
Control (no potash) plus 2 calcium carbonate	8.3			.101		
Do	9.6	8.3	18.6	.103	.100	.016
Do	7.2			.096		

Though the results are not as pronounced from cowpeas as with soybeans, the same general effect is produced Biotite and muscovite lead the insoluble minerals as carriers of potash. Orthoclase still seems to show a slight lead over microcline and where potash-carrying minerals were applied.

Lime has produced large increases where soluble potash is added, but its effect is not so great with the micaceous material as when soybeans were grown. This would indicate that, through the forces of weathering, a protective covering had formed around the particles of mica, preventing the plant roots from extracting as much potash as was done when the preceding legume was grown.

The rates of growth of cowpeas of the double applications of potash and lime are graphically given in figure 4.

Exceedingly small amounts of potash have been removed from the soil with the microline treatment. It gradually increases through treatments of orthoclase, muscovite, biotite until the maximum is reached with the soluble material (Pl. 49, D).

ACTIVE SOIL POTASH AFTER TWO YEARS' CROPPING

In order that the active or readily soluble soil potash left after two years' cropping might be determined, samples of each pot were subjected to solution in *N*/5 nitric acid. The samples were obtained by making three borings in each pot through the entire column of soil. The portions removed by each boring were well mixed, oven-dried, and bottled for analysis. Thirty-gm. portions were agitated with 500 cc. of *N*/5 nitric acid for 96 hours, after which the potash was determined colometrically. Table X gives the average amounts of potash removed by *N*/5 nitric acid.

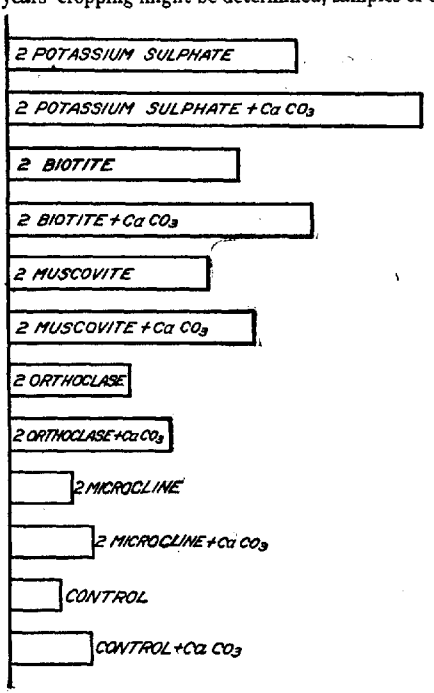

FIG. 4.—Rate of growth of cowpeas under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Considerably more potash was extracted by dilute acid when potassium sulphate had been added than when none had been used. Biotite and muscovite produced about the same quantity, more than double the amount of the controls. Very little, if any, increases could be discerned from the pots to which orthoclase or microcline had been applied.

Carbonate of lime does not have the slightest effect on any minerals used toward increasing the amount of potash soluble in *N*/5 nitric acid. In a majority of cases when the carbonate of lime has been used there are slight losses of potash from solution.

TABLE X.—*Active potash of soil after two years' cropping*

Treatment.	Potash carriers applied per pot.	Calcium carbonate applied per pot.	Potash recovered (p. p. m. of potassium oxid).	Gain or loss due to calcium carbonate.
	Gm.	*Gm.*		
Potassium sulphate	3.57	.....	34.7	.....
2 potassium sulphate	7.14	.....	55.9	.....
2 potassium sulphate plus calcium carbonate	7.14	16.71	53.2	−2.7
2 potassium sulphate plus 2 calcium carbonate	7.14	33.42	57.6	+1.7
Biotite	21.49	.....	24.5	.....
2 biotite	42.98	.....	32.6	.....
2 biotite plus calcium carbonate	42.98	16.71	26.6	−6.0
2 biotite plus 2 calcium carbonate	42.98	33.42	31.8	−0.8
Muscovite	19.87	.....	21.8	.....
2 muscovite	39.74	.....	35.9	.....
2 muscovite plus calcium carbonate	39.74	16.71	33.4	−2.5
2 muscovite plus 2 calcium carbonate	39.74	33.42	28.6	−7.3
Orthoclase	13.55	.....	13.2	.....
2 orthoclase	27.01	.....	15.2	.....
2 orthoclase plus calcium carbonate	27.01	16.71	12.5	−2.7
2 orthoclase plus 2 calcium carbonate	27.01	33.42	14.6	−0.6
Microcline	12.55	.....	13.0	.....
2 microcline	25.10	.....	14.8	.....
2 microcline plus calcium carbonate	25.10	16.71	10.8	−4.0
2 microcline plus 2 calcium carbonate	25.10	33.42	16.0	+1.2
Control (no potash)	.....	.....	12.9	.....
Control (no potash) plus calcium carbonate	.....	16.71	14.2	+1.3
Control (no potash) plus 2 calcium carbonate	.....	33.42	10.8	−3.4

SUMMARY

The chief points brought out by this investigation are as follows:

(1) Little difference in the solubility of potash in water is found among the common soil-forming minerals: Biotite, muscovite, orthoclase, and microcline.

(2) Biotite and muscovite give up considerably more of their potash to solutions of carbonic acid than do orthoclase or microcline. The order in which potash is removed by this solvent is biotite, muscovite, orthoclase, and microcline.

(3) Lime as calcium bicarbonate does not increase the solubility of potash in any of the above minerals.

(4) Pot experiments which include the growth of four crops—oats, soybean, rye, and cowpea—that have had potash supplied in the form of minerals show that these plants can extract different amounts of this element from them. Biotite is able to produce four times the amount of dry matter of oats as microcline and 66 per cent as much as potassium sulphate. Muscovite produces nearly twice as much dry matter as orthoclase. The same general effect is caused from these carriers of potash with rye.

(5) Lime in the form of precipitated carbonate has not materially increased the dry matter or the potash removed from the soil by oats or

rye. The dry matter of soybean has been increased about 33 per cent when lime was used in conjunction with biotite. There was also a noticeably increased growth from muscovite caused by calcium carbonate. A much smaller increase was found from this material when the potash was applied as orthoclase or microcline.

(6) Lime caused the soybeans to remove more potash from the soil with potassium-sulphate, biotite, and muscovite treatments. This should not be taken necessarily to indicate that potash has been driven into solution, but that more favorable conditions for plant growth have been set up in the soil. More vigorous plants are thus produced, plants capable of removing more of this nutrient material. The results from the cowpeas were similar to those of soybeans.

(7) Slightly more potash was removed, after two years' cropping, by $N/5$ nitric acid from the pots fertilized with biotite and muscovite than from the control pots. No more potash was removed by this solvent where orthoclase and microcline had been added than from the controls.

(8) Lime does not appear to increase the solubility of the soil potash in $N/5$ nitric acid from any of the treatments.

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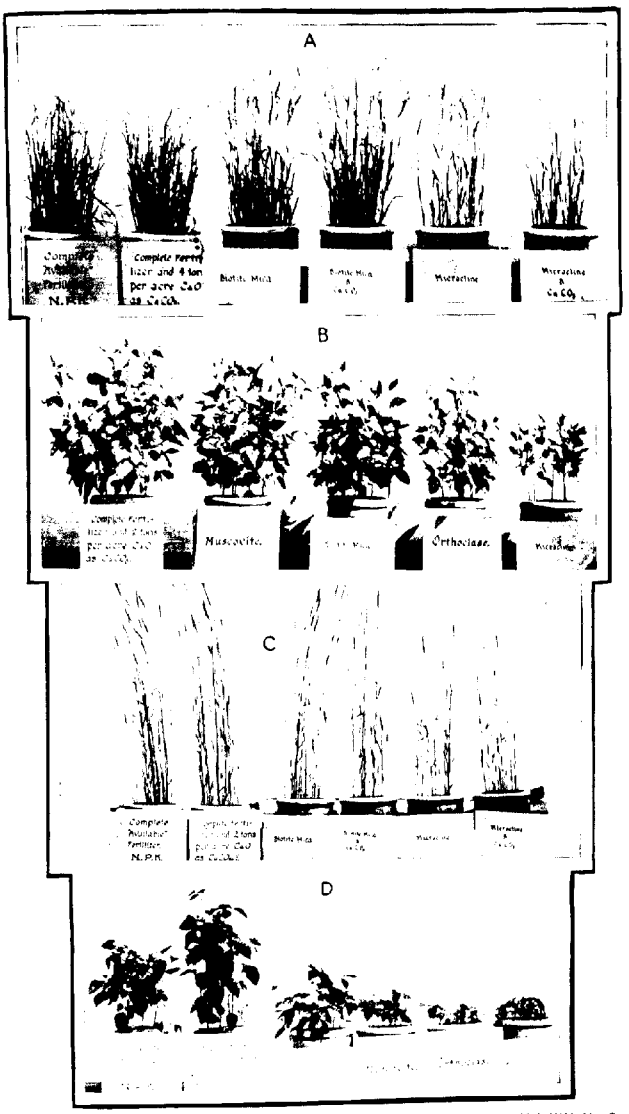
PLATE 49

A.—Oats, showing growth with potash from various minerals, with and without calcium carbonate.

B.—Soybeans, showing growth with potash from various minerals, with and without calcium carbonate.

C.—Rye, showing growth with potash from various minerals, with and without calcium carbonate.

D.—Cowpeas, showing growth with potash from various minerals, with and without calcium carbonate.


A
Complete Fertilizer and 4 ton per acre CaO as CaCO3
Biotite Mica
Microcline
B
Muscovite
Orthoclase
C
D

INFLUENCE OF REACTION ON NITROGEN-ASSIMILATING BACTERIA [1]

By E. B. FRED, *Agricultural Bacteriologist*, and AUDREY DAVENPORT, *Technical Assistant, Agricultural Experiment Station of the University of Wisconsin*

INTRODUCTION

One of the most powerful factors influencing the growth of legumes is the reaction of the soil. Indeed, it has been known for a long time that alfalfa, clover, and related plants will not thrive on an acid soil. Some idea of the importance of this problem may be seen from the vast amount of literature dealing with this subject which has appeared in recent years. These reports are concerned largely with the nature of the acid constituents of the soil and with their influence on the growth of the higher plant. Details of these investigations are not essential here, since this paper presents the results obtained in a study of the influence of acidity on bacteria rather than on higher plants. Because of the intimate relation between host plant and bacteria in the case of legumes, it seems that the results of these tests may be useful in explaining the influence of soil acidity on legumes.

A number of scientists have noted in a general way the effect of total acidity or alkalinity on the nodule-forming bacteria and on their host plants. Their results are of interest, but they fail to give information in regard to the proper reaction for the growth of the bacteria without the host plant. Repeatedly the question is asked, How long will the legume bacteria live in soil in the absence of the legume? Undoubtedly the answer to this question involves a study of many factors, among which reaction is important. It is the purpose in this work to establish the relation of *Rhizobium leguminosarum* from different plants to acid and to alkali.

Before presenting the results obtained a brief review of some of the previous contributions to this subject will be made.

HISTORICAL REVIEW

More than 30 years ago Beijerinck (*1*) [2] in his study of *Rhizobium leguminosarum* noted that this organism is injured in a medium of *N*/33.3 to *N*/50 concentration of acid. He found that a medium prepared from a decoction of pea stems, reaction *N*/166.6 malic acid, gave optimum conditions for growth.

From his observations Mazè (*17*) concluded that the legume bacteria may be divided into two great groups: (1) Those forms which are accus-

[1] Published by permission of the Director of the Wisconsin Agricultural Experiment Station.

[2] Reference is made by number (*italic*) to "Literature cited," p. 335-336.

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tomed to acid soils and (2) those forms accustomed to alkaline soils. He claimed that the legume bacteria of the alkaline-soil group may be so modified, if cultivated on acid media, that the organism will become adapted to an acid reaction and produce nodules on the acid-resistant legumes.

Süchting's (*22*) results indicate that the legume bacteria retain their infecting power better in a neutral than in an acid medium. From the results of pot tests, Moore (*18*) reported that the legume bacteria would stand any degree of acidity or alkalinity of the soil that would permit the growth of its particular legume. This investigator found that legume bacteria flourished in media which contained as high as 0.05 per cent or *N/128.04* of free citric acid. According to Maassen and Müller (*16*), the legume bacteria are very sensitive to the reaction of the medium.

In connection with a study of the factors that influence nodule formation, Zipfel (*26, p. 127*) noted that the legume bacteria were not very sensitive to small amounts of acid or alkali. According to Hiltner (*13*), the sensitiveness of lupines to liming is a result of the injurious effect of the lime on the nodule bacteria. Whiting (*24*) claims that *Rhizobium leguminosarum* is not very sensitive to the reaction of the medium. He agrees with Moore that in the soil, legume bacteria and their host plant are equally resistant to acids and alkalis. Prucha (*20*) studied the influence of varying concentrations of hydrochloric acid and sodium hydroxid on *Rhizobium leguminosarum* of alfalfa. He found that no growth occurred on agar slants containing 10 per cent of normal hydrochloric acid or a concentration of *N/10*, and that toward sodium hydroxid the alfalfa organisms were much more resistant, about 30 per cent of normal alkali, a concentration of *N/3.3* being required to inhibit the growth of the bacteria. The results of his experiments indicate that large amounts of acid or alkali inhibit the growth of the legume organism and interfere with its power to infect.

Morgan and Gruzit (*19*) reported that an acid reaction of *N/1,200* was toxic to the growth of soil bacteria, while *N/1,000* alkali was approximately the most suitable reaction. In a later report Gruzit (*11*) found that the soil bacteria were very sensitive to an acid reaction. Sulphuric acid in a concentration of *N/1,200* killed about 99.6 per cent of the soil flora; of *N/1,400* about 93 per cent; and *N/2,840* inhibited the growth of 42 per cent of the bacteria. On the other hand, the maximum number of bacteria was noted in solutions with a reaction of *N/1,000* alkaline. The author concluded that the soil bacteria were more sensitive to acidity than were the corn-plant seedlings. A decrease in the number and activity of the denitrifying bacteria and of Azotobacter and of *Rhizobium leguminosarum* in acid soils has been noted by Loew (*15*).

From this brief review of the literature it will be seen that the results do not agree. An explanation for this variation may be found in the

method of determining reaction. More recent study has shown that bacterial processes are influenced by the hydrogen-ion concentration of the medium rather than by the total acidity. Therefore it was planned in this study to measure true acidity, concentration of hydrogen ions, as well as total acidity of the culture media.

INFLUENCE OF ACIDITY AND ALKALINITY ON THE GROWTH OF BACTERIA

The data reported deal with the effect of varying reactions on the multiplication of bacteria from some of the more important legumes, as well as on the multiplication of two different strains of Azotobacter. The term "strain" as used in this paper is applied to the same species of an organism isolated from different sources; for instance, the writers studied eight strains of the alfalfa organism, all of which were separated in pure culture from plants grown in widely separated sections and from soils of different reaction.

IDENTIFICATION OF LEGUME BACTERIA

To prove that the organisms employed were pure and true to name, all cultures were replated at least twice before their general characteristics were studied. Table I shows some of the cultural characteristics of these microorganisms. On standard nutrient-agar slopes growth is moderate, at first colorless, later a faint brown. In standard gelatin stab, growth is slow, chiefly at the top of the medium, brownish in color, and no liquefaction is noted for one or two weeks; however, in older cultures, three months, the gelatin is slowly liquefied. No gas is produced from dextrose, lactose, or saccharose broths, although the media become cloudy, and frequently a white membrane is formed which covers the surface. The organisms grow slowly in nitrate broth without gas production. In neutral litmus milk no change is noted during the first week; but at the end of the second or third week this medium becomes alkaline, and the dye is partly reduced. After two weeks bromcresol-purple milk inoculated with the legume bacteria becomes alkaline. The difference between the legume bacteria of different plants and different strains is perhaps best demonstrated by the rate of growth on mannitol-agar stroke cultures. On this medium the legume bacteria may be divided according to their amount of growth into three groups: scanty, moderate, and abundant growers. The organisms from different sources show decided variations in their growth in standard nutrient broth. The presence or absence of a membrane however, seems to bear no relation to the growth on mannitol-agar slopes. Except in the media already described, the legume bacteria exhibit close agreement in their cultural characteristics.

TABLE I.—*Cultural and biochemical characteristics of legume bacteria after two weeks at 28° C.*

Name of organism.	Mannitol agar, surface growth.	Standard nutrient broth.		Litmus milk.		Bromcresol-purple milk reaction.
		Surface growth.	Clouding.	Reaction.	Reduction.	
Alfalfa 1	Abundant.	None	Turbid	Alkaline	Slight at bottom	Alkaline.
Alfalfa 2	Scanty	do	do	do	No reduction	Do.
Alfalfa 3	Abundant.	Membranous.	do	do	do	Do.
Alfalfa 4	do	None	do	do	Slight at bottom	Do.
Alfalfa 5	do	Membranous.	do	do	do	Do.
Alfalfa 6	do	None	do	do	No reduction	Do.
Alfalfa 7	do	do	do	do	do	No change.
Alfalfa 8	Moderate	do	do			
Sweet clover 9	Abundant.	do	do	Alkaline	Slight at bottom	Alkaline.
Garden pea 10	Moderate.	do	do	do	No reduction	Do.
Field pea 11	do					
Vetch 12	do	None	Turbid	Alkaline	No reduction	Do.
Red clover 13	Abundant.	Membranous.	do	do	No reduction; slimy at top.	Do.
Red clover 14	do					
Common bean 15	Scanty					
Soy bean 16	do					
Soy bean 17	Abundant.	Membranous.	Turbid	Alkaline	No reduction; slimy at top.	Do.
Velvet bean 18	Moderate.					
Lupine 19	Scanty	None		Alkaline	None	Do.
Lupine 20	Abundant.	do	Turbid	do	Slight at bottom	Do.
Lupine 21	do	Membranous.	do	do	No reduction; slimy at top.	Do.
Lupine 22	do	do	do	do	do	Do.
Lupine 23	Scanty	None	do	do	No reduction	Do.

PRODUCTION OF NODULES

The final test of identity of a pure culture of the legume bacteria consisted in the formation of nodules on the legume from which the culture was obtained. For this purpose the leguminous plant and the microorganism were grown in large Pyrex tubes containing agar, under conditions which excluded all other forms of life. When nodules developed, a new isolation was made from the nodule and the organism secured in this way was compared with the original culture. In several cases these new cultures were again tested under sterile conditions for nodule formation. The lupines failed to grow well in the large test tubes, and for this legume a mixture of sterilized sand and soil was used. In every case the organism caused the formation of nodules, while the roots of the control plants were entirely free of nodules.

STAINING CHARACTERISTICS

The bacteria from the nodule or from agar slopes stain readily with carbol-fuchsin, gentian-violet, or methylene-blue. Perhaps the best preparations were obtained from the use of carbol-fuchsin, followed by a slight decolorization with dilute alcohol. The organism is Gram-negative when ethyl alcohol is used in the decolorizing process.

The number of flagella seems to depend on the source of the culture or on its age. This point, however, deserves more careful study. The fol-

lowing strains of alfalfa (*Medicago sativa*) and lupine (*Lupinus* sp.) were stained for flagella:

Alfalfa 1, 5, 6, 7, 8, peritrichous flagella.

Lupine 19, single, or rarely two, flagella.

The shape and general structure of the flagella of the lupine organism were different from those of the alfalfa organism. For instance, the flagella of lupine 19 are not so long and wavy as those of alfalfa.

EXPERIMENTAL PROCEDURE

It was realized from the beginning that the success of this study depended largely on the number of parallel tests and the number of different strains of bacteria employed. Therefore each experiment was repeated several times. In order to have comparable results all of the organisms were grown on the same medium, the composition of which is given below:

Mannitol ($C_6H_8(OH)_6$)	10.0 gm.
Magnesium sulphate ($MgSO_4+7H_2O$)	0.2 gm.
Dibasic potassium phosphate (K_2HPO_4)	0.2 gm.
Sodium chlorid (NaCl)	0.2 gm.
Calcium sulphate ($CaSO_4+2H_2O$)	0.1 gm.
Distilled water	1,000.0 cc.

Only the purest chemicals and conductivity water were used in preparing the culture medium. The reaction of the medium was usually neutral to phenolphthalein, although in some of the tests a very small amount of alkali was required to make it neutral. After dividing this culture solution among a series of flasks, the portions were sterilized and adjusted to different reactions with *N/10* or *N/20* acid and alkali. The normality of the culture medium is shown in the tabular data.

In beginning the experiments the acid and alkali limits of growth as determined by previous investigators were tried, and repeated tests were made until the critical point for the growth of the particular organism was reached.

Because of the importance of the acid-soil problem and the use of legumes in an acid system of agriculture, the greater part of this paper is concerned with the relation of legume bacteria to acidity, while their relation to alkalinity has received only limited study. With the exception of certain preliminary experiments, consideration was given not only to the total quantity of acid added but also to the effect of this acid on altering the hydrogen-ion concentration of the medium. Because of the low content of buffer substances in the mannitol medium, only small quantities of sulphuric acid were required to alter its hydrogen-ion concentration.

To determine the nature and extent of the buffer effect, preliminary tests of the hydrogen-ion concentration of the culture medium were made. For this purpose, a series of flasks of the medium was prepared in such

a way that one of its constituents was omitted from each test and the hydrogen-ion concentration measured immediately after the addition of the acid or alkali. It was stated in a previous paper that possibly the mannitol exerts a buffer effect. However, later investigations do not support this statement. The concentration of hydrogen ions in

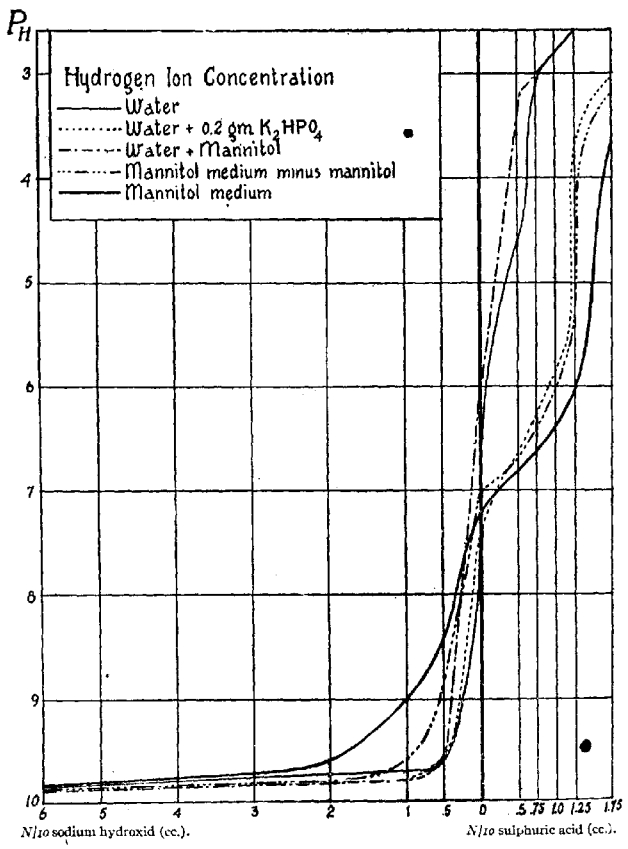

FIG. 1.—Graphs showing the buffer effect of the various constituents of mannitol medium.

pure water to which varying amounts of acid and alkali were added, as well as the concentration in the synthetic culture medium plus acid and alkali, is shown in figure 1. It appears from the graphs that the dibasic potassium phosphate is the chief buffer substance and that the mannitol has little effect on the concentration of hydrogen ions.

The concentration of hydrogen ions was measured by the colorimetric method as outlined by Clark and Lubs (*6*). The procedure was as follows: To a 10-cc. portion of the culture fluid the proper indicator was added, and the color developed was compared with the colors obtained on the addition of the same indicator to tubes of 10 cc. of the various "buffer solutions" of known hydrogen-ion concentration. Since the accuracy of this method depends on the standard "buffer solutions," these were prepared from chemicals purified as directed by Clark and Lubs, and the buffer mixtures checked by the electrometric method. In every case the two methods of measuring the hydrogen-ion exponent gave almost identical results. All of the data are reported as the hydrogen-ion exponent or P_H, instead of in terms of the normality of hydrogen ions. In the alkaline range, especially where large amounts of the base were used, the concentration of hydroxyl ions was frequently beyond the range of the indicators. Therefore the exponent of the hydrogen ion in the presence of large amounts of alkali is not correct.

INFLUENCE OF SULPHURIC ACID AND SODIUM HYDROXID ON THE REPRODUCTION OF NITROGEN-ASSIMILATING BACTERIA

TOTAL ACID AND ALKALI

A considerable number of experiments were made with *Rhizobium leguminosarum* from different plants and in general the agreement between these tests was good; therefore only a few of the typical ones are presented. The data show that the legume bacteria vary in their resistance to acidity, depending on the source of the organism. Taylor (*23*) has shown very clearly that acids, especially organic acids, vary in their degree of activity in checking the growth of bacteria, but that the inorganic acids, hydrochloric and nitric, however, show much similarity of action. In this work no attempt was made to try out different acids or alkalis, but rather to measure the action of sulphuric acid and of sodium hydroxid.

EXPERIMENTS WITH ALFALFA BACTERIA

In the following experiments a 1-cc. suspension of legume bacteria in sterilized water was used to inoculate 100 cc. of mannitol medium (p. 321) in 750-cc. Erlenmeyer flasks. At regular intervals of one week each the cultures were shaken vigorously and 1 cc. removed for plate counts. It was noticed early that increasing the acidity of a medium had a decided effect on the growth of the bacteria. Thus, the least acid members of a series were the first to show turbidity, while the more acid the reaction, the longer the period required for a noticeable turbidity to appear. The results obtained are given in Table II. In mannitol culture medium the injurious effect of alkali on legume bacteria is not noticeable unless added in amounts greater than *N/125*, while all growth is prevented in

N/62.5 sodium hydroxid. Toward gram-equivalent amounts of sulphuric acid these organisms behave differently; it seems that sulphuric acid is approximately 10 times as toxic to the bacteria as sodium hydroxid of the same normality. Growth is retarded in concentrations of *N/1,000*, and the cells are killed in solutions of the concentration of *N/500* sulphuric acid. These results do not agree with those of Beijerinck, who found a reaction of *N/166.6* acid gave optimum growth for *Rhizobium leguminosarum*. However, this difference in behavior of the bacteria no doubt is due to the difference in culture medium. From a glance at the data of this table it is plain that *R. leguminosarum* is much more sensitive to sulphuric acid than to gram-equivalent amounts of sodium hydroxid.

TABLE II.—*Effect of sulphuric acid and sodium hydroxid on the reproduction of alfalfa bacteria, strain I*

No.	Normal acid or alkali in 100 cc. of medium	Concentration of acid or alkali.	Number of bacteria in 1 cc. of medium.					
			Inoculum.	After 1 week.	After 2 weeks.	After 3 weeks.	After 4 weeks.	After 6 weeks.
1	Neutral.....	Neutral....	35,000	10,640,000	25,900,000	55,600,000	53,200,000	83,800,000
2	0.05 cc. sulphuric acid.	*N/2,000*....	35,000	9,000,000	21,520,000	44,600,000	32,500,000	54,900,000
3	0.1 cc. sulphuric acid.	*N/1,000*....	35,000	7,010,000	19,580,000	44,300,000	31,000,000	19,400,000
4	0.2 cc. sulphuric acid.	*N/500*.....	35,000	None.	None.	None.	None.	None.
5	0.3 cc. sulphuric acid.	*N/333*.....	35,000	None.	None.	None.	None.	None.
6	0.1 cc. sodium hydroxid.	*N/1,000*....	35,000	7,000,000	22,280,000	42,900,000	29,200,000	65,500,000
7	0.2 cc. sodium hydroxid.	*N/500*.....	35,000	5,070,000	18,280,000	31,600,000	30,100,000	12,500,000
8	0.4 cc. sodium hydroxid.	*N/250*.....	35,000	3,750,000	13,220,000	23,600,000	29,800,000	28,500,000
9	0.8 cc. sodium hydroxid.	*N/125*.....	35,000	7,110,000	12,540,000	26,600,000	21,900,000	23,600,000
10	1.6 cc. sodium hydroxid.	*N/62.5*.....	35,000	None.	None.	None.	None.	None.

A comparison of the effect of treatment on the number of bacteria at various intervals of time failed to show any decided difference. In relation to time, the acid or alkali exerted approximately the same effect on the multiplication of bacteria after one or six weeks.

EXPERIMENTS WITH AZOTOBACTER, ALFALFA, LUPINE, RED CLOVER, AND SOYBEAN BACTERIA

The behavior of alfalfa bacteria is in accord with our knowledge of the host plant—that is, they are sensitive to acidity. The question which naturally suggests itself is that of the relation of other strains of legume bacteria to different reactions. In addition to the legume bacteria, one strain of Azotobacter was studied. Only one count, two weeks after inoculation, was made, since the number of organisms at different intervals of time had failed to show any marked variation. The averages of the plate counts after two weeks are given in Table III. The results with alfalfa confirm those of the earlier tests and show that this organism is

killed quickly in solutions containing small amounts of acid. The difference in behavior of the bacteria from alfalfa and lupine is evident. The latter are more resistant to sulphuric acid than the former.

TABLE III.—*Effect of sulphuric acid and sodium hydroxid on the reproduction of nitrogen-fixing bacteria*

No.	Normal acid or alkali in 100 cc. of medium.	Concentration of acid or alkali.	Number of bacteria in 1 cc. of medium two weeks after inoculation.				
			Alfalfa 1 (inoculum 350,000).	Lupine 21 (inoculum 1,570,000).	Red clover 13 (inoculum 1,300,000).	Soybean 17 (inoculum 1,100,000).	Azotobacter 131.
1	Neutral	Neutral	12,300,000	30,000,000	17,100,000	29,100,000	1,560,000
2	0.1 cc. sulphuric acid	*N*/1,000	7,300,000	21,900,000	Lost.	22,300,000	212,000
3	0.2 cc. sulphuric acid	*N*/500	None.	700	Lost.	None.	None.
4	0.5 cc. sulphuric acid	*N*/200	None.	None.	None.	None.	None.
5	0.1 cc. sodium hydroxid	*N*/1,000	11,900,000	24,300,000	15,600,000	21,900,000	7,120,000
6	0.2 cc. sodium hydroxid	*N*/500	9,800,000	26,100,000	17,800,000	22,300,000	3,820,000
7	0.5 cc. sodium hydroxid	*N*/200	Lost.	11,500,000	22,700,000	15,100,000	None.
8	1.0 cc. sodium hydroxid	*N*/100	8,600,000	5,900	12,000,000	7,100,000	None.

One very striking fact shown in the data of this experiment is the narrow limits of growth of Azotobacter. This organism is readily affected by small amounts of acid or alkali, the limits of growth are approximately *N*/1,000 acid and *N*/500 alkali. These data are in agreement with the results of previous investigators. For instance, it has been shown by Christensen and his associates (*3*, *4*, *5*) that the formation of Azotobacter film in mannitol cultures inoculated with soil is correlated with the reaction of the soil—that is, acid soils fail to show any film.

From the data of the previous tests no conclusions can be drawn with respect to the acid or alkali limit of growth of bacteria except within a relatively wide range. Therefore further tests were arranged in such a way as to give a series of cultures of varying concentration of acid and alkali. Here the difference between the reactions of the cultures was less than in former experiments. Instead of counting the total number of bacteria at different intervals, the cultures were incubated for 21 days and then tested for the presence or absence of living bacteria. The turbidity of the culture was noted, the presence of bacteria determined as shown by a stained mount, and mannitol-agar slants were inoculated with a loop of the cultures. The presence or absence of growth on the agar slants was taken as an indication of the presence or absence of living bacteria in the culture.

A comparison of the development of different legume bacteria in media of varying reactions is presented in Table IV. The data here reported were obtained from a series of separate tests. The greater resistance of the lupine bacteria to acidity as compared with the alfalfa bacteria is clearly shown by the results presented in this table. Seven different strains of the alfalfa organism and four of lupine were studied, and in each test these different strains of the alfalfa and lupine organism behaved alike. The acid range for alfalfa bacteria is approximately

N/909 and for lupine bacteria *N/588*. The resistance of the lupine bacteria to acids is in accord with the results of analyses of the root juices. Lemmermann (*14*) has shown that the extract from roots of lupines is more acid than that from roots of beans, peas, vetch, or serradella.

TABLE IV.—*Effect of sulphuric acid on the reproduction of legume bacteria after 21 days.*

No.	Normal sulphuric acid in 100 cc. of medium.	Concentration of acid.	Result after 21 days.						
			Alfalfa 1.	Alfalfa 2.	Alfalfa 3.	Alfalfa 4.	Alfalfa 5.	Alfalfa 7.	Alfalfa 8.
1	*Cc.* Neutral	Neutral	Growth	Growth	Growth	Growth	Growth	Growth	Growth
2	0.025	*N*/4,000	do	do	do	do	do	do	Do.
3	0.050	*N*/2,000	do	do	do	do	do	do	Do.
4	0.072	*N*/1,389	do	do	do	do	do	do	Do.
5	0.075	*N*/1,333	do	do	do	do	do	do	Do.
6	0.080	*N*/1,250	do	do	do	do	do	do	Do.
7	0.088	*N*/1,136	do	do	do	do	do	do	Do.
8	0.092	*N*/1,087	do	do	do	do	do	do	Do.
9	0.096	*N*/1,042	do	do	do	do	do	do	Do.
10	0.100	*N*/1,000	do	do	do	do	do	do	Do.
11	0.110	*N*/909	None	None	None	None	None	None	None.
12	0.120	*N*/833	do	do	do	do	do	do	Do.
13	0.125	*N*/800	do	do	do	do	do	do	Do.
14	0.130	*N*/769	do	do	do	do	do	do	Do.
15	0.132	*N*/757	do	do	do	do	do	do	Do.
16	0.135	*N*/741	do	do	do	do	do	do	Do.
17	0.140	*N*/714	do	do	do	do	do	do	Do.
18	0.145	*N*/690	do	do	do	do	do	do	Do.
19	0.150	*N*/667	do	do	do	do	do	do	Do.
20	0.156	*N*/641	do	do	do	do	do	do	Do.
21	0.160	*N*/625	do	do	do	do	do	do	Do.
22	0.168	*N*/595	do	do	do	do	do	do	Do.
23	0.170	*N*/588	do	do	do	do	do	do	Do.
24	0.180	*N*/556	do	do	do	do	do	do	Do.
25	0.190	*N*/526	do	do	do	do	do	do	Do.

No.	Normal sulphuric acid in 100 cc. of medium.	Concentration of acid.	Result after 21 days.					
			Sweet clover 9.	Garden pea 10.	Field pea 11.	Vetch 12.	Red clover. 13.	Red clover 14.
1	*Cc.* Neutral	Neutral	Growth	Growth	Growth	Growth	Growth	Growth.
2	0.025	*N*/4,000	do	do	do	do	do	Do.
3	0.050	*N*/2,000	do	do	do	do	do	Do.
4	0.072	*N*/1,389	do	do	do	do	do	Do.
5	0.075	*N*/1,333	do	do	do	do	do	Do.
6	0.080	*N*/1,250	do	do	do	do	do	Do.
7	0.088	*N*/1,136	do	do	do	do	do	Do.
8	0.092	*N*/1,087	do	do	do	do	do	Do.
9	0.096	*N*/1,042	do	do	do	do	do	Do.
10	0.100	*N*/1,000	do	do	do	do	do	Do.
11	0.110	*N*/909	None	None	None	None	None	None.
12	0.120	*N*/833	do	do	do	do	do	Do.
13	0.125	*N*/800	do	do	do	do	do	Do.
14	0.130	*N*/769	do	do	do	do	do	Do.
15	0.132	*N*/757	do	do	do	do	do	Do.
16	0.135	*N*/741	do	do	do	do	do	Do.
17	0.140	*N*/714	do	do	do	do	do	Do.
18	0.145	*N*/690	do	do	do	do	do	Do.
19	0.150	*N*/667	do	do	do	do	do	Do.
20	0.156	*N*/641	do	do	do	do	do	Do.
21	0.160	*N*/625	do	do	do	do	do	Do.
22	0.168	*N*/595	do	do	do	do	do	Do.
23	0.170	*N*/588	do	do	do	do	do	Do.
24	0.180	*N*/556	do	do	do	do	do	Do.
25	0.190	*N*/526	do	do	do	do	do	Do.

TABLE IV.—*Effect of sulphuric acid on the reproduction of legume bacteria after 21 days*—Continued

No.	Normal sulphuric acid in 100 cc. of medium.	Concentration of acid.	Result after 21 days.					
			Velvet bean 18.	Soy-bean 17.	Lupine 19.	Lupine 20.	Lupine 21.	Lupine 22.
	Cc.							
1	Neutral	Neutral	Growth	Growth	Growth	Growth	Growth	Growth.
2	0.025	*N*/4,000	do	do	do	do	do	Do.
3	0.050	*N*/2,000	do	do	do	do	do	Do.
4	0.072	*N*/1,380	do	do	do	do	do	Do.
5	0.075	*N*/1,333	do	do	do	do	do	Do.
6	0.080	*N*/1,250	do	do	do	do	do	Do.
7	0.088	*N*/1,130	do	do	do	do	do	Do.
8	0.092	*N*/1,087	do	do	do	do	do	Do.
9	0.096	*N*/1,042	do	do	do	do	do	Do.
10	0.100	*N*/1,000	do	do	do	do	do	Do.
11	0.110	*N*/909	do	do	do	do	do	Do.
12	0.120	*N*/833	do	do	do	do	do	Do.
13	0.125	*N*/800	do	do	do	do	do	Do.
14	0.130	*N*/769	do	do	do	do	do	Do.
15	0.132	*N*/757	do	do	do	do	do	Do.
16	0.135	*N*/741	do	do	do	do	do	Do.
17	0.140	*N*/714	do	do	do	do	do	Do.
18	0.145	*N*/690	do	do	do	do	do	Do.
19	0.150	*N*/667	do	do	do	do	do	Do.
20	0.156	*N*/641	do	do	do	do	do	Do.
21	0.160	*N*/625	None.	None.	do	do	do	Do.
22	0.168	*N*/595	do	do	do	do	do	Do.
23	0.170	*N*/588	do	do	None.	None.	do	Do.
24	0.180	*N*/556	do	do	do	do	None.	None.
25	0.190	*N*/526	do	do	do	do	do	Do.

Because of the large number of cultures used and the small difference in amount of acid between each culture it is possible to separate the legume bacteria into classes, depending on their resistance to acidity. If grouped in this way, the alfalfa organism would stand at the alkaline end of the scale, the lupine organism at the acid end. Sweet clover, vetch, garden pea, red clover, velvet bean, and soybean organisms would occupy an intermediate position and about in the order named, graded from acid sensitive to acid resistant.

In the case of lupine and alfalfa, the different strains of the same organism show remarkable agreement and support the statement that the influence of acid on the lower plant, *Rhizobium leguminosarum*, is similar to the influence of acid on the higher plant, the legume.

EXPERIMENTS WITH AZOTOBACTER

The selection of Azotobacter was prompted by the fact that various investigators have reported that the growth of this organism may be used as an indicator of the reaction of soil. In order to test the influence of acid and alkali on Azotobacter, a series of mannitol cultures was prepared and treated as given in the preceding experiments. Three separate tests were made and the data recorded in Table V. In every culture in which acid or alkali was used a very marked effect on growth was noted. When compared with the legume organism regardless of the source, it is plain that Azotobacter is much more sensitive to changes in reaction.

Apparently the acid limit for Azotobacter is about *N/1,333.3* and the alkaline limit of growth about *N/1,000*. In relation to nitrates, Azotobacter behaves in a somewhat similar manner—that is, it is more sensitive to high concentrations than is *Rhizobium leguminosarum* (*12, p. 209*).

TABLE V.—*Effect of sulphuric acid and sodium hydroxid on the reproduction of Azotobacter*

No.	Normal acid or alkali in 100 cc. of medium.	Concentration of acid or alkali.	Result after 21 days.		
			Azotobacter 130.	Azotobacter 131.	Azotobacter 130.
1	Neutral		Growth	Growth.	Growth.
2	0.025 cc. sulphuric acid	*N/4,000*	....do...	....do...	Do.
3	0.050 cc. sulphuric acid	*N/2,000*	....do...	....do...	Do.
4	0.075 cc. sulphuric acid	*N/1,333*	....do...	....do...	Trace.
5	0.100 cc. sulphuric acid	*N/1,000*	None...	None...	None.
6	0.110 cc. sulphuric acid	*N/909*	....do...	....do...	Do.
7	0.120 cc. sulphuric acid	*N/833*	....do...	....do...	Do.
8	0.125 cc. sulphuric acid	*N/800*	....do...	....do...	Do.
9	0.050 cc. sodium hydroxid	*N/2,000*	Growth	Growth.	
10	0.100 cc. sodium hydroxid	*N/1,000*	....do...	....do...	
11	0.200 cc. sodium hydroxid	*N/500*	None...	Trace...	
12	0.500 cc. sodium hydroxid	*N/200*	....do...	None...	

INFLUENCE OF SULPHURIC ACID AND SODIUM HYDROXID ON THE REPRODUCTION OF NITROGEN-ASSIMILATING BACTERIA

DISSOCIATED ACID OR ALKALI

The marked influence of reaction upon the nitrogen-fixing bacteria, especially certain strains of the legume organisms and Azotobacter, has been pointed out in the results of this paper. The evidence submitted is sufficient to warrant the conclusion that sulphuric acid in mannitol solution is more toxic than is the hydrogen equivalent amount of sodium hydroxid. This difference in the action of acid and alkali may be due in part to their difference in dissociation. Attention was called to this point in an earlier paper (*10*).

EXPERIMENTS WITH ALFALFA AND LUPINE BACTERIA

In Table VI are given the hydrogen-ion exponents for each of 16 culture solutions and the growth of the organisms as shown by transfers to agar slopes. The cultures are arranged in order of decreasing acidity and the reaction of the culture medium varies as shown in the table from P_H 4.6 to P_H 9.8; the readings for the high alkaline range are not absolute. A study of the data shows that the alfalfa bacteria are more sensitive to true acidity than are the lupine bacteria. The acid limit of growth as shown in this test is between P_H 5.4 and P_H 6.0 for alfalfa and lower than P_H 4.6 for lupine. In all cases there was a good agreement between the growth of the different strains of the same organism.

TABLE VI.—*Effect of the concentration of hydrogen ions on the reproduction of alfalfa and lupine bacteria*

No.	P_H	Result after 21 days.					
		Alfalfa 6.	Alfalfa 3.	Alfalfa 5.	Lupine 19.	Lupine 19.	Lupine 21.
1	4.6	.....	None	None	.....	Growth	Growth.
2	5.4	None	.....	.....	Growth	.....	
3	6.0	Growth	Growth	Growth	do	Growth	Do.
4	6.2	do	.....	.....	do	.....	
5	6.4	.....	Growth	Growth	.....	Growth	Do.
6	6.6	Growth	do	do	Growth	do	Do.
7	6.8	do	do	do	do	do	Do.
8	7.4	do	do	do	do	do	Do.
9	8.4	do	do	do	do	do	Do.
10	8.6	do	.....	.....	do	.....	
11	8.8	do	.....	.....	do	.....	
12	9.0	.....	Growth	Growth	do	Growth	Do.
13	9.2	Growth	.....	.....	None	.....	
14	9.4	do	.....	.....	do	.....	
15	9.6	.....	Growth	Growth	.....	Growth	Do.
16	9.8	.....	do	None	None	None	None.

A second experiment was set up similar to the preceding except that only the acid range was tested. Twenty-one strains of legume bacteria were studied. All the data for this test are summarized in Table VII. An examination of the results shows clearly that the growth of the legume bacteria in culture solutions of varying reactions is proportional to the hydrogen-ion concentration of the medium, and it is probable that their difference in resistance to hydrogen ions is related to the reaction of the sap of the host plant. As shown in the data of Tables VI and VII, the organisms of alfalfa are the most sensitive of the legume bacteria to the concentration of hydrogen ions, while the lupine bacteria are the most resistant. In relation to true acidity, the sweet-clover, garden-pea, field-pea, vetch, common-bean, red-clover, soybean, and velvet-bean organisms occupy a position between alfalfa and lupine bacteria. The velvet-bean and the soybean organisms show considerable resistance to an increase in hydrogen-ion concentration.

It is of interest to note the results obtained by other investigators. Brünn (*2*) found that *Bacillus coli* is killed within 24 hours if exposed to an acid reaction of $P_H = 4.7$, but not of $P_H = 5.0$. Wolf and Harris (*25*) reported that the difference between the reaction which just permits growth and the reaction which prevents growth is not great. They suggest the term "critical P_H" which is obtained by taking the average of the two values, the P_H which just permits growth and the P_H which inhibits growth. They found that the critical reaction for *Bacillus welchii* (*B. perfringens*) is about $P_H = 4.82$ and for *B. sporogenes* (Metchnikoff) about $P_H = 4.94$. In both tests glucose peptone media were used.

TABLE VII.—*Effect of concentration of hydrogen ions on the reproduction of legume bacteria after 21 days*

No.	PH.	Result after 21 days.						
		Alfalfa 1.	Alfalfa 2.	Alfalfa 3.	Alfalfa 4.	Alfalfa 5.	Alfalfa 7.	Alfalfa 8.
1	3.0	None.	None.	None.	None.	None.	None.	None.
2	3.1	do.	do.	do.	do.	do.	do.	Do.
3	3.2	do.	do.	do.	do.	do.	do.	Do.
4	3.4	do.	do.	do.	do.	do.	do.	Do.
5	3.5	do.	do.	do.	do.	do.	do.	Do.
6	3.6	do.	do.	do.	do.	do.	do.	Do.
7	3.7	do.	do.	do.	do.	do.	do.	Do.
8	3.8	do.	do.	do.	do.	do.	do.	Do.
9	4.0	do.	do.	do.	do.	do.	do.	Do.
10	4.1	do.	do.	do.	do.	do.	do.	Do.
11	4.3	do.	do.	do.	do.	do.	do.	Do.
12	4.6	do.	do.	do.	do.	do.	do.	Do.
13	4.8	do.	do.	do.	do.	do.	do.	Do.
14	5.0	Growth.	Growth.	Growth.	Growth.	Growth.	Growth.	Growth.
15	5.2	do.	do.	do.	do.	do.	do.	Do.
16	5.4	do.	do.	do.	do.	do.	do.	Do.
17	5.5	do.	do.	do.	do.	do.	do.	Do.
18	5.6	do.	do.	do.	do.	do.	do.	Do.
19	5.7	do.	do.	do.	do.	do.	do.	Do.
20	5.9	do.	do.	do.	do.	do.	do.	Do.
21	6.1	do.	do.	do.	do.	do.	do.	Do.
22	6.2	do.	do.	do.	do.	do.	do.	Do.
23	6.3	do.	do.	do.	do.	do.	do.	Do.
24	6.4	do.	do.	do.	do.	do.	do.	Do.
25	6.6	do.	do.	do.	do.	do.	do.	Do.
26	6.8	do.	do.	do.	do.	do.	do.	Do.
27	7.0	do.	do.	do.	do.	do.	do.	Do.
28	7.1	do.	do.	do.	do.	do.	do.	Do.

No.	PH.	Result after 21 days.						
		Sweet clover 9.	Garden pea 10.	Field pea 11.	Vetch 12.	Red clover 14.	Red clover 13.	Bean 15.
1	3.0	None.	None.	None.	None.	None.	None.	None.
2	3.1	do.	do.	do.	do.	do.	do.	Do.
3	3.2	do.	do.	do.	do.	do.	do.	Do.
4	3.4	do.	do.	do.	do.	do.	do.	Do.
5	3.5	do.	do.	do.	do.	do.	do.	Do.
6	3.6	do.	do.	do.	do.	do.	do.	Do.
7	3.7	do.	do.	do.	do.	do.	do.	Do.
8	3.8	do.	do.	do.	do.	do.	do.	Do.
9	4.0	do.	do.	do.	do.	do.	do.	Do.
10	4.1	do.	do.	do.	do.	do.	do.	Do.
11	4.3	do.	do.	do.	do.	Growth.	Growth.	Growth.
12	4.6	do.	do.	do.	do.	do.	do.	Do.
13	4.8	Growth.	Growth.	Growth.	Growth.	do.	do.	Do.
14	5.0	do.	do.	do.	do.	do.	do.	Do.
15	5.2	do.	do.	do.	do.	do.	do.	Do.
16	5.4	do.	do.	do.	do.	do.	do.	Do.
17	5.5	do.	do.	do.	do.	do.	do.	Do.
18	5.6	do.	do.	do.	do.	do.	do.	Do.
19	5.7	do.	do.	do.	do.	do.	do.	Do.
20	5.9	do.	do.	do.	do.	do.	do.	Do.
21	6.1	do.	do.	do.	do.	do.	do.	Do.
22	6.2	do.	do.	do.	do.	do.	do.	Do.
23	6.3	do.	do.	do.	do.	do.	do.	Do.
24	6.4	do.	do.	do.	do.	do.	do.	Do.
25	6.6	do.	do.	do.	do.	do.	do.	Do.
26	6.8	do.	do.	do.	do.	do.	do.	Do.
27	7.0	do.	do.	do.	do.	do.	do.	Do.
28	7.1	do.	do.	do.	do.	do.	do.	Do.

TABLE VII.—*Effect of concentration of hydrogen ions on the reproduction of legume bacteria after 21 days*—Continued

No.	P_H	Result after 21 days.						
		Soy-bean 16.	Soy-bean 17.	Velvet bean 18.	Lupine 19.	Lupine 20.	Lupine 21.	Lupine 22.
1	3.0	None....	None....	None....	None....	None....	None....	None.
2	3.1	...do....	...do....	...do....	...do....	...do....	...do....	Do.
3	3.2	...do....	...do....	...do....	Growth.	Growth.	Growth.	Growth.
4	3.4	...do....	Growth.	Growth.	...do....	...do....	...do....	Do.
5	3.5	Growth.	...do....	...do....	...do....	...do....	...do....	Do.
6	3.6	...do....	...do....	...do....	...do....	...do....	...do....	Do.
7	3.7	...do....	...do....	...do....	...do....	...do....	...do....	Do.
8	3.8	...do....	...do....	...do....	...do....	...do....	...do....	Do.
9	4.0	...do....	...do....	...do....	...do....	...do....	...do....	Do.
10	4.1	...do....	...do....	...do....	...do....	...do....	...do....	Do.
11	4.3	...do....	...do....	...do....	...do....	...do....	...do....	Do.
12	4.6	...do....	...do....	...do....	...do....	...do....	...do....	Do.
13	4.8	...do....	...do....	...do....	...do....	...do....	...do....	Do.
14	5.0	...do....	...do....	...do....	...do....	...do....	...do....	Do.
15	5.2	...do....	...do....	...do....	...do....	...do....	...do....	Do.
16	5.4	...do....	...do....	...do....	...do....	...do....	...do....	Do.
17	5.5	...do....	...do....	...do....	...do....	...do....	...do....	Do.
18	5.6	...do....	...do....	...do....	...do....	...do....	...do....	Do.
19	5.7	...do....	...do....	...do....	...do....	...do....	...do....	Do.
20	5.9	...do....	...do....	...do....	...do....	...do....	...do....	Do.
21	6.1	...do....	...do....	...do....	...do....	...do....	...do....	Do.
22	6.2	...do....	...do....	...do....	...do....	...do....	...do....	Do.
23	6.3	...do....	...do....	...do....	...do....	...do....	...do....	Do.
24	6.4	...do....	...do....	...do....	...do....	...do....	...do....	Do.
25	6.6	...do....	...do....	...do....	...do....	...do....	...do....	Do.
26	6.8	...do....	...do....	...do....	...do....	...do....	...do....	Do.
27	7.0	...do....	...do....	...do....	...do....	...do....	...do....	Do.
28	7.1	...do....	...do....	...do....	...do....	...do....	...do....	Do.

The limit of growth and critical P_H values for the legume bacteria and Azotobacter are about as follows:

No.	Organism.	Acid value of P_H which allows growth.	Acid value of P_H which inhibits growth.	Mean value of P_H or the critical P_H.
1	*Rhizobium leguminosarum* from alfalfa	5.0	4.8	4.9
2	*Rhizobium leguminosarum* from sweet clover	5.0	4.8	4.9
3	*Rhizobium leguminosarum* from garden pea	4.8	4.6	4.7
4	*Rhizobium leguminosarum* from field pea	4.8	4.6	4.7
5	*Rhizobium leguminosarum* from vetch	4.8	4.6	4.7
6	*Rhizobium leguminosarum* from red clover	4.3	4.1	4.2
7	*Rhizobium leguminosarum* from bean	4.3	4.1	4.2
8	*Rhizobium leguminosarum* from soybean	3.4	3.2	3.3
9	*Rhizobium leguminosarum* from velvet bean	3.4	3.2	3.3
10	*Rhizobium leguminosarum* from lupine	3.2	3.1	3.15
11	Azotobacter	6.6	6.4	6.5

If the critical P_H value of the legume bacteria be compared with the growth of the leguminous plant in soil of varying reaction, it will be noted that the bacteria in relation to acidity behave similar to their host plants. Here, then, is a characteristic of the legume bacteria which separates these organisms into different groups, acid sensitive, acid resistant, and no doubt a long list of organisms intermediate between the two extremes.

EXPERIMENTS WITH AZOTOBACTER

The general plan followed was similar to that outlined in Table V. The results obtained are given in Table VIII. Here, again, the extreme sensitiveness of Azotobacter to true acid or alkali is plainly shown. The limit of hydrogen-ion concentration for the growth of this organism is about P_H 6.5. In agreement with the results of the previous experiments it is clear that toward hydrogen ions Azotobacter is more sensitive than any of the legume bacteria used in this investigation. The narrow limits of growth for Azotobacter, P_H 6.6 to 8.4 or 8.8, indicate that the growth of this organism may be used to measure the reaction of various substances.

TABLE VIII.—*Effect of the concentration of hydrogen ions on the reproduction of Azotobacter*

No.	P_H.	Azotobacter 130.	Azotobacter 131.	Azotobacter 130.
1	4.6	.....	None.....	None.
2	5.4	None.....	.....do.....	Do.
3	6.0	.....do.....	.....do.....	Do.
4	6.2	.....do.....	.....	Do.
5	6.4	.....	None.....	Do.
6	6.6	None.....	Growth.....	Growth.
7	6.8	Growth.....	.....do.....	Do.
8	7.4	.....do.....	.....do.....	Do.
9	8.4	.....do.....	.....do.....	
10	8.6	None.....	.....	
11	8.8	.....do.....	Growth.....	
12	9.0	.....do.....	.....	
13	9.2	.....	.....	
14	9.4	None.....	.....	
15	9.6	.....	None.....	
16	9.8	.....	.....do.....	

TABLE IX.—*Effect of Rhizobium leguminosarum and of Azotobacter on the reaction of the culture medium*

Name of organism.	P_H value.			Name of organism.	P_H value.		
	Beginning.	End.	Difference.		Beginning.	End.	Difference.
Alfalfa 1.....	7.2	7.1	0.1	Garden bean 15....	7.2	6.9	0.3
Alfalfa 2.....	7.2	7.0	.2	Cowpea 28.....	7.2	7.1	.1
Alfalfa 3.....	7.2	7.0	.2	Vetch 12.....	7.2	7.0	.2
Alfalfa 4.....	7.2	7.0	.2	Field pea 11.....	7.2	7.0	.2
Alfalfa 7.....	7.2	6.9	.3	Garden pea 24.....	7.2	6.9	.3
Alfalfa 8.....	7.2	7.0	.2	Garden pea 25.....	7.2	7.0	.2
Sweet clover 9.....	7.2	7.0	.2	Garden pea 26.....	7.2	6.8	.4
Sweet clover 29....	7.2	7.0	.2	Serradella 27.....	7.2	7.1	.1
Red clover 13.....	7.2	7.0	.2	Lupine 19.....	7.2	7.2	.0
Red clover 14.....	7.2	6.8	.4	Lupine 20.....	7.2	7.1	.1
Soybean 16.....	7.2	7.1	.1	Lupine 22.....	7.2	7.0	.2
Soybean 17.....	7.2	7.0	.2	Lupine 21.....	7.2	7.1	.1
Velvet bean 18.....	7.2	7.2	.0	Azotobacter 131....	7.2	5.1	2.1

INFLUENCE OF NITROGEN-ASSIMILATING BACTERIA ON THE REACTION OF THE MEDIUM

Since the legume bacteria show a difference in behavior toward reaction of the culture medium, it was thought that the growth of the different strains might cause a noticeable variation in the reaction of the medium. Accordingly, the reaction was measured by titrating the cultures with *N/20* acid or alkali at the time of inoculation and again four weeks later. The results of titrations failed to show any decided change in the reaction of the culture medium after the growth of the different organisms, although there was a slight increase in acidity. Similar results were reported in an earlier publication (9).

The results of hydrogen-ion measurements of the inoculated and uninoculated culture solutions showed a small but distinct increase in acidity. In this test saccharose solution was used in place of the mannitol. In Table IX only the averages of duplicate cultures are given. As a rule, the change in the reaction due to the growth of *R. leguminosarum* in the saccharose solution was from P_H 0.1 to 0.4, the average about P_H 0.2. This gain in acidity is very small when compared with that produced by Azotobacter—namely, 2.1. Because of the turbidity of the culture medium, which is caused by the great number of bacteria, it seems strange that there is only a slight change in the hydrogen-ion concentration. Determinations of the amount of sugar consumed by these organisms in liquid media offer an explanation for the small increase in acid. It has been found that *R. leguminosarum* may develop in enormous numbers without consuming more than 4 to 5 per cent of the total amount of sugar in the medium (9).

SUMMARY

The behavior of the legume bacteria as well as Azotobacter toward small amounts of acid or alkali depends upon many factors: Chief among these are the nature of the medium and the dissociation of the acid and alkali.

All the results point to the fact that *R. leguminosarum* regardless of strain, does not persist for any length of time in a medium, the reaction of which prevents reproduction.

In these experiments, which were arranged to study the influence of reaction on the nitrogen-assimilating bacteria, 21 strains of *R. leguminosarum* and two of Azotobacter were studied. In general, *R. leguminosarum* showed similar cultural characteristics—that is, bacteria from different legumes. The most noticeable difference was that of rate of growth certain strains developing much more rapidly than others. On the ordinary culture media *R. leguminosarum* does not show any very characteristic growth. The identity of the

organism was studied for each strain and in every case the organism used to inoculate plants grown under sterile conditions effected inoculation.

In all of the tests the organisms were inoculated into 50-cc. portions of mannitol solution in 200-cc. Erlenmeyer flasks, the reaction changed by the addition of sulphuric acid or sodium hydroxid, and the cultures incubated for four weeks at 28° C. At the end of the period of incubation the presence or absence of the bacteria was determined by plate counts, microscopical mounts, and by inoculation of mannitol-agar slants. Aside from the total acid or alkali, the hydrogen-ion content in these cultures was measured by the colorimetric method.

The results of these experiments show clearly that sulphuric acid in culture solutions is far more injurious to alfalfa bacteria than to lupine bacteria. In other words, the nodule bacteria from different plants behave differently toward acid. The legume bacteria may be divided into groups about as follows:

1. Critical P_H 4.9.....Alfalfa and sweet clover.
2. Critical P_H 4.7.....Garden pea, field pea, and vetch.
3. Critical P_H 4.2.....Red clover and common beans.
4. Critical P_H 3.3.....Soybeans and velvet beans.
5. Critical P_H 3.15.....Lupines.

The alfalfa organism is the most sensitive of the legume bacteria to acidity, and, conversely, the lupine organism is the most resistant to acidity.

The toxicity of sodium hydroxid toward legume bacteria is not noticeable until the alkali is added in large amounts; approximately 10 times as much normal alkali as normal acid is required to produce a similar injury. The organisms from the nodules of different legumes failed to show any decided difference in respect to alkali. For instance, it appears that the alkali limit of growth is the same for *Rhizobium leguminosarum* from lupine or from alfalfa.

One striking fact noted in the data of these experiments is the extreme sensitiveness of Azotobacter to slight changes in reaction. As compared with the legume bacteria, this organism is far more sensitive. The acid limit of growth in mannitol solution for Azotobacter is about *N/1,333.3* and the alkaline limit about *N/1,000*, or the critical P_H acid value 6.5 and the alkaline value 8.6.

In relation to hydrogen-ion concentration of medium the nodule bacteria from different legumes show a very decided difference. The evidence supports the conclusion that a correlation exists between the acid resistance of the bacteria and the acid resistance of the higher plant.

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